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UNIVERSITY OF ALBERTA

INVESTIGATIONS FOR IMPROVEMENT OF LACTOSE ABSORPTION FROM DAIRY FOODS BY LACTOSE INTOLERANT INDIVIDUALS

BY

(C)

NAGENDRA P. SHAH

A THESIS
SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY
IN
FOOD PROCESSING

DEPARTMENT OF FOOD SCIENCE

EDMONTON, ALBERTA

SPRING, 1991

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Date : Nov 30, 1990.

To my dad, **Amichand**, and mom, **Fulmati Devi**.
To my wife, **Nirmala**
To my mentor, **Pavel Jelen**

ABSTRACT

The overall aim of this project was to find a suitable way for the incorporation of whey to foods for consumption by lactose intolerant populations. In order to assess the magnitude of lactose intolerance problem, a survey of several ethnic groups typical of the Nepalese population was conducted which indicated a high prevalence of lactose intolerance in some ethnic groups.

The purpose of the second study was to ascertain whether the microorganisms or their lactases can survive low pH conditions such as found in the human stomach. Lactase activities and survival of lactic acid bacteria (LAB) and of their ß-galactosidases under acidic conditions were studied. Cultures of *Lactobacillus delbrueckii* subsp. *bulgaricus* exhibited the highest ß-galactosidase activity among the LAB studied. Viable counts of all LAB decreased rapidly at lower pH levels, but *L. acidophilus* survived better than the other organisms. The decrease of enzyme activity of intact cultures at pH 3.5 was slight, however, acidification of sonicated culture to this pH resulted in rapid and permanent loss of enzyme activity.

The hypothesis was to test if foods containing higher solids would result in better tolerance of lactose than foods with lower solids. This led to the study of yogurt, acidophilus and buttermilk cultures in quarg processing and the partitioning patterns of these culture organisms into quarg and the resulting whey. The acidophilus culture was found to be unsuitable for quarg processing. After whey separation, the concentration of bacteria from all three cultures were higher in the quarg than in the whey.

The quargs and wheys were used for the next experiment to evaluate the lactose malabsorption effects of solid and liquid foods using post-weaning rats. The animals were fed yogurt (Y), quargs prepared from yogurt culture (QY) and buttermilk culture (QBM), and two types of whey (WY and WBM) obtained from quarg processing, for a week. Blood glucose assays and absence of diarrhea indicated that Y, QY, and QBM were well tolerated. Wheys containing similar levels of viable culture organisms and lactose as the quargs caused severe symptoms of diarrhea. Plate counts and enzyme assays of gastrointestinal contents confirmed the presence of viable organisms and ß-galactosidase activity after feeding the two types of quarg.

The results from rat studies were confirmed using lactase-deficient human subjects. Four lactose malabsorbers consumed 250 g of yogurt, QY or QBM, pasteurized quarg (QP), and WY containing 15 g lactose after an overnight fast. Blood glucose was measured and symptoms of lactose intolerance were recorded following consumption of various products. QY was well tolerated. QBM and QP were similarly tolerated as yogurt. WY caused symptoms of gastrointestinal discomfort in all the subjects. Results indicated that microbial enzyme activity or presence of viable culture organisms are not necessary for lactose digestion and that food consistency seemed to be an important factor for efficient digestion and absorption of lactose. This suggests that whey can be incorporated into foods suitable for consumption by lactose intolerant people as long as the solid contents of the foods are kept high. The results of the study led to the concept of whey-based solid food which was, in an informal trial, found to be suitable for lactose malabsorbers.

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1. INTRODUCTION

1.1. BACKGROUND INFORMATION

"Lactose intolerance" is a condition in which lactose is not hydrolysed into its constituent monosaccharides by the ß-galactosidase enzyme located in the small intestine of lactase-deficient individuals. This leads to gastrointestinal symptoms such as bloating, cramps, and diarrhea which result from the fermentation of unhydrolysed lactose by the colonic microflora (Rosensweig, 1969; Kretchmer, 1972; Asp and Dahlqvist, 1972; Houts, 1988).

Studies have demonstrated high incidence of lactose intolerance among Africans, Asians, and American Negroes in contrast to low prevalence in Caucasians of Scandinavian and northern European origin (Davis and Bolin, 1972, Kleyn, 1972; Reddy and Pershad, 1972; Scrimshaw and Murray, 1988). The high prevalence of lactose intolerance among non-Caucasian populations could be attributed to low milk production and consumption in these areas. It is estimated that approximately 70% of the world's population is lactose intolerant (Simoons, 1978; Savaiano and Levitt, 1987).

Lactase deficient individuals are unable to properly digest and utilize milk. This has important implications for human nutrition. Milk is an excellent source of protein, calcium and other nutrients such as vitamin B12, riboflavin, etc. Lactase deficient persons avoid milk and other dairy products high in calcium because of the malabsorption of lactose and the accompanying lactose intolerance symptoms and therefore may consume less calcium than required. Although the absorption of other nutrients contained in milk is not affected in lactose malabsorbers (Debongnie et al., 1979), ingestion of milk may cause diarrhea resulting in nutrient deficiencies in general (Brown et al., 1980; Gurr, 1987; Leichter, 1981; Leichter et al., 1984; Turner et al., 1976).

Lactose-hydrolysed milk has been suggested for lactose-intolerant consumers (Alm, 1982; Shah, 1985; Gurr, 1987). Lactase-deficient individuals can obtain nutrients from lactose-hydrolysed milk without any symptoms. The nutritional consequences of lactase treated milk and long term acceptance of low lactose milk have been discussed by many researchers (Barillas and Solomons, 1987; Biller et al., 1987; Cheng et al., 1979; McCormick, 1976; Payne et al., 1977; Paige et al., 1975).

Fermented dairy products are commonly used in the areas of the world where lactose intolerance is prevalent. Yogurt appears to be tolerated by lactose intolerant individuals (Alm, 1982; Kolars et al., 1984; Savaiano et al., 1984). The mechanism of lactose digestion from yogurt is not completely understood. Baer (1970) suggested that better tolerance of yogurt is due to reduction in lactose content during fermentation. However it should be noted that commercial yogurt often contains more lactose than milk as nonfat dry milk is added to the milk to increase the viscosity of the product and only a small portion of the lactose is hydrolysed during fermentation. Thus it appears that there may be other factors responsible for better tolerance of yogurt.

One school of thought is that the culture organisms used in the manufacture of yogurt survive the gastric digestion and the enzymes liberated from the bacteria are responsible for efficient digestion of lactose in lactose malabsorbers.

"Dahi", a fermented milk product similar to yogurt, is popular in Nepal and is used as an important part of the indigenous diet. Dahi may be similarly tolerated as yogurt as it is processed using similar types of organisms.

Tolerance of lactose by lactose intolerant individuals from other cultured dairy products, such as quarg, cottage cheese, or labneh, has not been studied so far. Quarg is a fermented dairy product with higher total solid contents than yogurt. A product similar to flavored quarg known as "Shrikhand" is popular in India, particularly in the states of Gujarat, Maharastra and Karnataka. Shrikhand is prepared by lactic acid coagulation of milk and draining of whey from the curd followed by mixing of sugar and flavoring. Labneh (leben), a concentrated yogurt of around 24% total solids popular in the Middle East, is processed by partial whey removal from yogurt (Tamime and Robinson, 1978). The product technology is similar to that of quarg.

1.2. UTILIZATION OF WHEY

Whey contains 6.5% total solids of which lactose (70-80%) and proteins (9-11%) are the major constituents (Kosikowski, 1979). Table 1-1 shows the average composition of sweet and acid wheys. There is more lactic acid, calcium, and phosphorus in acid whey as compared to sweet whey (Cox, 1973; Kosikowski, 1977). Whey has high biological oxygen demand and therefore is regarded as a serious pollutant. In recent years scarcity of milk and strict regulations regarding disposal of whey have prompted the dairy industry to look at ways to utilize the valuable nutrients from whey.

The cheese industry is growing rapidly in Nepal and there have been no efforts made towards utilization of whey (Shah, personal experience). This could lead to a similar problem of whey utilization as in other cheese producing countries. However, in a country with problems of malnutrition (National Nutrition Coordination Committee, 1979), the whey should be utilized as a valuable human nutrient source rather than dumping it down the hills. Also, much whey should be available

Table 1-1. Average composition of sweet and acid type of wheys [a]

	Sweet whey (pH 5.9-6.4)	Acid whey (pH 4.6-4.7)
	---------------%	--------------
Total solids	6.3- 7.0	6.3- 7.0
Protein	0.6- 0.8	0.6- 0.7
Lactose	4.6- 5.2	4.4- 4.6
Fat	0.02- 0.01	0.02- 0.05
Calcium	0.04- 0.05	0.13- 0.16
Phosphorus	0.1- 0.3	0.2- 0.45
Potassium	0.14- 0.16	0.14- 0.16
Citrate	0.14- 0.16	0.14- 0.17
Chloride	0.1	0.09
Lactic acid	0.2	0.64

[a] adapted from Glass and Hedrick (1976a); Glass and Hedrick (1976b); Jelen (1979); Kosikowski (1979).

internationally as an inexpensive nutrient source for starving populations.

Nutritive value of whey is high. It is a good source of calcium and phosphorus. Nutritional composition, vitamin, mineral, and calorie contents of sweet and acid type of dry wheys have been reported by many researchers (Glass and Hedrick, 1976a; Glass and Hedrick, 1976b; Jelen, 1979; Kosikowski, 1979). Whey protein is one of the highest quality proteins. Whey solids are utilized in many foods including infant formulations, ice creams, cheese spreads, bakery and confections (Mathur and Shahani, 1979). Whey has been used successfully in beverages (Holsinger et al., 1974). Products such as ricotta cheese utilize only the whey protein, leaving behind lactose, a valuable source of energy, and the pollution problem unsolved (Jelen, 1979; Shah, 1986). However, as the major constituent in whey is lactose, incorporation of whey to foods would make it difficult to be used by the lactose intolerant people.

There are several alternatives which could lead to utilization of whey in Nepal. Whey can be used as a drink if lactose in the whey is hydrolysed by adding lactase enzyme from microbial sources. However, the commercially available enzymes are expensive and are not available in the developing countries. Whey can be used in making a dahi-like product. Use of whey for making mysost cheese requires evaporation of water from the whey which could be very expensive.

1.3. RESEARCH OBJECTIVES

The principal objective was to find a suitable way for the utilization of whey as a source of nutrients for formulated human foods in a country where there may be a large incidence of lactose intolerance, such as in Nepal. The overall research plan was to conduct a survey to estimate the prevalence of lactose intolerance in various ethnic groups of Nepal; to ascertain if the culture microorganisms or their ß-galactosidases survive acidic conditions; to study the feasibility of quarg processing using yogurt, acidophilus, and buttermilk cultures and to prepare quargs and whey with similar number of bacteria; to use quargs and whey to evaluate the lactose malabsorption effects of solid and liquid foods using rats; and finally to confirm the results from rat studies in lactose intolerant human subjects.

The purposes were to study whether the microorganisms or their ß-galactosidases or both are responsible for efficient digestion of lactose in lactose malabsorbers; to investigate if whey containing similar levels and types of microorganisms as yogurt could be similarly tolerated as yogurt; and to test if foods containing higher solids (such as quarg) can result in efficient digestion and absorption of lactose.

Specific objectives were:

(1) to study the magnitude of lactose intolerance problems in different ethnic groups constituting the Nepalese population;

(2) to determine the ß-galactosidase activities of *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Streptococcus salivarius* subsp. *thermophilus*, *Lactobacillus acidophilus*, and *Lactococcus lactis* subsp. *cremoris*, to study the properties of crude lactases isolated from these organisms, and to determine whether lactic acid bacteria (LAB) and their lactases can survive low pH conditions usually found in human stomach;

(3) to compare the use of yogurt, acidophilus, and buttermilk cultures in quarg production and to assess the partitioning of these culture organisms into quarg and the resulting whey;

(4) to evaluate the lactose malabsorption effects of a solid (such as quarg) and liquid (such as quarg cheese whey) dairy foods in post-weaning rats and to ascertain the survival levels of the microorganisms and/or their ß-galactosidase activity in rats;

(5) to verify the findings of rat studies in lactase-deficient human subjects and to study the relative importance of the type of culture, food consistency, and exogenous enzyme activity in the dairy products in alleviating the lactose intolerance symptoms; and

(6) to propose a conceptual approach for use of whey in a model Nepali food suitable for lactose intolerant populations.

1.4. EXPERIMENTAL INVESTIGATIONS, METHODOLOGY, RESULTS AND DISCUSSION

The body of this study, presented after the overall literature review in chapter 2, consists of 5 manuscripts of which three have already been published, one is in print and one has been submitted for publication, all in refereed journals. In addition, a patent application has been filed resulting from a work related to lactase activity and properties of sonicated dairy cultures.

The experimental work was divided as follows: (1) study to determine the magnitude of lactose intolerance problem in different ethnic groups of Nepal based on a questionnaire; (2) study of survival of lactic acid bacteria and their lactases under acidic conditions; and lactase activity and properties of sonicated dairy cultures; (3) evaluation of rennet effects and partitioning of microbial cultures in quarg cheese manufacture; (4) evaluation of lactose absorption effects by post-weaning rats from yogurt, quarg and quarg whey; and (5) study of food consistency effects in lactose absorption by lactose intolerant individuals from yogurt, quarg, pasteurized quarg, and quarg whey.

Each of the five research areas is presented in a separate paper which contains descriptions of materials and methods, results and discussion, accompanied by pertinent tables, graphs, conclusions, and bibliographies. The format of each chapter follows the publication requirements of the various journals. The footnote at the first page of each chapter shows names of authors involved in the investigation and the journal concerned.

The survey of households (chapter 3) representative of Nepalese populations to determine the magnitude of lactose intolerance problems was conducted in the Chitwan district of Nepal. The rat feeding studies (chapter 6) were performed in the Animal

Science Department, University of Alberta, and the experiments involving human subjects (chapter 7) were carried out at the Clinical Investigation Unit, the University of Alberta Hospitals. Acknowledgements to personnel who assisted with these studies are included in the appropriate chapters.

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2. REVIEW OF LITERATURE

2.1. LACTOSE INTOLERANCE

Lactose, a disaccharide composed of the monosaccharides glucose and galactose, occurs only in mammalian milk. The milk of certain pacific sea mammals such as seals, sea lions, and walruses is devoid of lactose (Kretchmer, 1972; Friedl, 1981). Cow's milk contains approximately 4.5-5.0% lactose as compared with 7.0-7.5% in human milk (Savaiano and Levitt, 1987; Scrimshaw and Murray, 1988). Upon ingestion, lactose is hydrolysed by a ß-galactosidase (lactase) enzyme located on the brush border of epithelial cells of the small intestine (Asp and Dahlqvist, 1972; Kretchmer, 1972; Houts, 1988; Bayless and Paige, 1979; Solomons, 1986; Nuys, 1980; Sandine and Daly, 1979; Newcomer, 1979). After hydrolysis, glucose and galactose are actively absorbed into the blood stream (Friedl, 1981; Hourigan and Mittal, 1984; Lebenthal et al., 1975; Francis, 1978; McDonald, 1978). In lactase deficient individuals when the amount of ingested lactose exceeds the splitting capacity of the available intestinal lactase, a portion of the ingested lactose remains unaltered. The undigested lactose passes from the small intestine to the colon where water is drawn from the tissues by osmotic action and fermentation by colonic microflora produces organic acid and gas (hydrogen and carbon dioxide). The large amount of water drawn into the intestine and the products of fermentation are largely responsible for the symptoms of lactose intolerance such as bloating, flatulence, cramps, abdominal pain, borborygmi, and diarrhea (Kleyn, 1972; Kretchmer, 1972; Reasoner et al., 1981; Burgio et al., 1984; Garza and Scrimshaw, 1976; Katy and Speckmann, 1978; National Dairy Council, 1985; Whelan and Stare, 1980; Rosado et al., 1987; Newcomer and McGill, 1984; Hurt, 1972; Fitzgerald, 1976).

2.2. TERMS RELATED TO DIGESTION AND ABSORPTION OF LACTOSE

The following terms have been proposed by the Protein Advisory Group (PAG) of the United Nations (Hourigan and Mittal, 1984).

2.2.1. Lactase Deficiency (Low Lactase Activity or Hypolactasia)

This term is used to indicate low intestinal lactase activity. A person, who shows a blood glucose rise of less than 20 mg/dL following ingestion of a standard lactose dose of 50 g, is classified as lactase deficient (Bolin and Davis, 1970; Friedl, 1981).

The lactase deficiency can be congenital, primary or secondary Welsh, 1981). The congenital lactase deficiency is a condition in which the lactase is absent from the intestinal mucosa at birth and is a rare occurence. In primary lactase deficiency, the lactase activity decreases in adulthood and only about 5-10% of original lactase activity remains. This form of lactase deficiency is genetically controlled. The secondary type of lactase deficiency implies a reduction in intestinal lactase activity which may result from malnutrition or a disease process such as gastroenteritis and is usually temporary. Approximately 70% of the world's population is afflicted with primary lactase deficiency (Simoons, 1978; Savaiano and Levitt, 1987).

2.2.2. Lactose Malabsorption

This refers to incomplete digestion and absorption of lactose as a consequence of lactase deficiency. Primary lactase deficiency leads to lactose malabsorption. This condition is simply one's inability to hydrolyse some or all of ingested lactose as a result of lactase deficiency. Lactose malabsorption can be diagnosed by blood glucose tests or breath hydrogen tests following ingestion of lactose.

2.2.3. Lactose Intolerance

An individual, who experiences symptoms of gastrointestinal discomfort such as bloating, cramps or diarrhea after ingesting lactose, is classified as lactose intolerant. Not all lactose malabsorbers show symptoms of lactose intolerance. There are varying degrees of tolerance among lactose malabsorbers (Friedl, 1981; Hourigan and Mittal, 1984; Savaiano and Levitt, 1987).

2.3. INCIDENCE OF LACTOSE INTOLERANCE

Studies of intestinal enzyme development in rats indicate that the ß-galactosidase activity in the intestinal cells increases during the prenatal period, remains relatively high throughout infancy, and then decreases after weaning (Johnson, 1981). Fig 2-1 shows intestinal lactase activity in the rat during various phases of development.

All mammals show a similar pattern of enzyme activity. Table 2-1 illustrates lactase activity in the intestine at different stages of growth. As shown in the Table 2-1, a small amount of ß-galactosidase activity is left in the adult stage. Pig, calf, rabbit, cat, and dog show similar patterns of enzyme activity as the rat (Kretchmer, 1972; Johnson, 1981).

In the majority of the world's population, primary lactase deficiency is common. Caucasians from northern European ethnic background and some African and Indian tribes maintain high intestinal lactase activity throughout life and can consume milk without any gastrointestinal discomfort (Kretchmer, 1972; Simoons, 1978; Johnson, 1981; Alm, 1982).

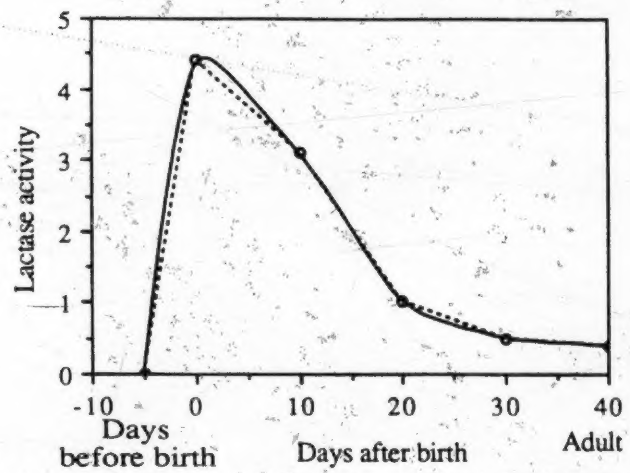

Fig 2-1. Beta-galactosidase activity of the intestine of the developing rat (from Kretchmer, 1972; Johnson, 1981).

Table 2-1. Lactase activity of the intestine in relation to the suckling phase of growth [a]

	Units of activity per gram of material						
Phase	Rat	Pig	Calf	Rabbit	Guinea Pig	Cat	Dog
At birth	18.0	18.0	50.0	14.0	5.0	4.0	6.0
At end of suckling period	2.0	2.0	11.0	5.0	4.0	-	-
Adult	2.0	1.0	0.2	0.3	4.0	0.5	0.7

[a] adapted from Johnson (1981)

All the population groups living in the non-dairying areas of the world have a high incidence of lactose intolerance (Simoons, 1981; Cavalli-Sforza et al., 1987; Segal et al., 1983). As milk production in these areas is scarce, adult population consume very little milk. On the other hand, populations with low incidence of lactose intolerance come from dairying areas of the world (Simoons et al., 1977).

Two hypotheses have been proposed regarding the incidence of lactose intolerance. The adaptation theory suggests that people who continue to consume milk or other milk products containing lactose beyond weaning period produce lactase. The presence of milk, thus lactose, stimulates lactase activity. Those who don't consume milk regularly may become lactase deficient (Johnson, 1981). Based on animal (Bolin et al., 1969) and human studies (Kretchmer, 1971; Johnson, 1981; Keusch et al., 1969), it appears that lactase deficiency is irreversible.

Genetic theory indicates that intestinal lactase activity level after weaning in humans is a heritable characteristic (Johnson, 1981; Simoons, 1981; Newcomer, 1978). This hypothesis is commonly accepted. The populations of the world who developed dairying and have accepted milk and milk products as a part of their diet are lactose tolerant. These people belong to western and eastern European ancestry and dairying people of Africa and south Asia (Friedl, 1981). It is estimated that this selection process began approximately 10, 000 years ago (Simoons, 1981).

Many review articles have been published on the prevalence of lactose intolerance (Paige and Bayless, 1981; Hourigan and Mittal, 1984; Scrimshaw and Murray, 1988). The magnitude of lactose intolerance is high (50-100%) in non-Caucasian populations of the world, in contrast to a low incidence (0-30%) in Caucasians, as shown in Fig 2-2.

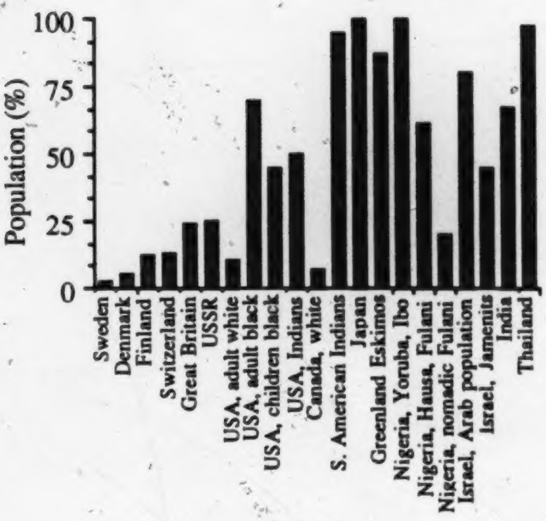

Fig 2-2. Frequency of lactose intolerance in some parts of the world (adapted from Alm, 1982; Kretchmer, 1972).

2.4. LACTOSE INTOLERANCE IN SOUTH EAST ASIA

Studies have indicated that there is a high prevalence of lactose intolerance in Asia (Davis and Bolin, 1972; Banerjee, 1972; Rosensweig, 1969; Segal et al., 1983; Chang et al., 1987). Rosensweig (1969) claims that the incidence of lactase deficiency is about 95% for Asians. The level of lactose intolerance has been reported to be 97-98% for Thais, 90% among Chinese and 67% among Indians (Rosensweig, 1969; Alm, 1982; Kretchmer, 1972). Brown et al. (1979) studied the prevalence of lactose intolerance among Bangladeshi children and found that over 80% of the children over 3 years of age were afflicted with this problem. Tandon et al. (1981) investigated the incidence of lactose malabsorption in south and north India and reported that about 19-50 % of north and 67% of south Indians were lactose intolerant. Desai et al. (1970) and Gupta et al., (1970) challenged 64 and 15 Indian subjects respectively, with standard lactose dose of 50 g and observed that all the subjects were lactase deficient as measured by breath hydrogen and showed symptoms of lactose intolerance. Reddy and Pershad (1972) estimated an incidence of lactose intolerance of 61% for Indians. Kar and Tandon (1985) ascertained the magnitude of lactase deficiency in Nagaland state of India and observed a high prevalence of lactose intolerance. Mittal et al. (1979) administered 250 mL of milk (equivalent to 12.5 g lactose) to 7 Indian people and reported that all showed symptoms with such a small amount of milk. Jeejeebhoy et al. (1964) performed lactose tolerance test on 32 Indian adult patients. Thirty one people of 32 (97%) showed symptoms of lactose malabsorption and their blood glucose did not rise > 20 mg/dL. Senewirajwe et al. (1977) measured breath hydrogen of 145 Sri Lankan subjects in a lactose tolerance test and observed that 73% of the subjects were affected with the problems of lactose intolerance. There have been no reports from other parts of south east Asia including Nepal regarding the magnitude of problems of lactose intolerance. The ethnic groups of Nepal are diverse. Brahmins have long history of milk usage whereas Tharus are non-dairying people. The incidence of lactose intolerance in Tharu ethnic group could be expected to be higher.

2.5. SCREENING TECHNIQUES FOR LACTOSE MALABSORPTION

2.5.1. Blood Tests

Plasma glucose. Lactase deficiency in individuals can be detected by administering 50 g of lactose in water and checking blood glucose level every 15 min for up to 2 h. An elevation of plasma glucose < 20 mg/dL after ingestion of lactose is indicative of lactase deficiency (Solomons, 1981; Newcomer and McGill, 1984). Lactose malabsorbers can not hydrolyze all the lactose and their blood glucose level does not rise.

Plasma galactose. This test involves administration of an oral dose of 50 g lactose and alcohol. Ethanol inhibits conversion of galactose to glucose in the liver. For this test, blood sample is taken at 15 min intervals for 45 min. An individual showing a plasma galactose rise < 5 mg/dL is classified as a lactose malabsorber (Solomons, 1981).

This method is more sensitive than blood glucose method, however, alcohol administration may not be suitable in all subjects.

2.5.2. Breath Hydrogen Test (BHT)

The breath hydrogen test is based on the fact that when a lactose malabsorber consumes milk or a lactose containing dairy product, the intact lactose passes from the small intestine to the colon where it is fermented into volatile fatty acids by normal human colonic flora; this fermentation process typically results in production of hydrogen gas. About 14 to 21% of the hydrogen produced is expired by the lungs and a rise in breath hydrogen of > 20 ppm is indicative of lactose intolerance (Solomons, 1981; Winter, 1987). There is a stoichiometric relationship between the amount of lactose fermented and hydrogen formed by the colonic flora (Bolin and Davis, 1970;

Fernandes et al., 1978; Solomons et al., 1980; Solomons, 1981; Flatz et al., 1984; Welsh et al., 1981).

2.5.3. Other Tests

Blood and BHT are acceptable tests. Many other tests, such as lactase assay by intubation methods, radiographic techniques, fecal analysis, etc. as outlined by Solomons (1981), are used for lactose malabsorption. Analysis of signs and symptoms, such as flatulence, abdominal cramps, bloating and diarrhea, after lactose tolerance test can be used as a supplementary evidence for diagnosis of lactose malabsorption.

2.6. TOLERANCE OF LACTOSE FROM A LIQUID LACTOSE SOLUTION VERSUS OTHER DAIRY PRODUCTS

Lactose tolerance test (LTT) is based on a dose of 50 g of lactose in water. This amount of lactose is present in about one litre of milk. Several studies have shown that whole milk can be better tolerated than skim milk and skim milk can be better tolerated than aqueous lactose solution (Leichter, 1973; Hourigan and Mittal, 1984; Savaiano and Levitt, 1987). The fat in whole milk delays gastric emptying (Leichter, 1981). Skim milk contains no fat; however, protein in skim milk is responsible for slower gastric emptying as compared to a lactose solution (Savaiano and Levitt, 1987). Liquid lactose solution, which is traditionally used for lactose intolerance test, would pass faster in the gastrointestinal tract as compared to that of skim milk or whole milk, therefore, the intensity of symptoms in lactase deficient individuals would be the highest with aqueous lactose solution (Leitchter, 1981; Savaiano and Levitt, 1987; Cavalli-Sforza and Strata, 1987).

Welsh and Hall (1977) measured gastric emptying times in lactose absorbers and malabsorbers after feeding glucose or lactose solutions, unflavored milk or chocolate milk. Both lactose absorbers and malabsorbers showed similar emptying times. However, chocolate milk, which had higher osmolality than the unflavored milk or lactose or glucose solution, emptied more slowly.

Nguyen et al. (1982) observed marked differences in amounts of breath hydrogen excreted with different kinds of milk. Nine lactose intolerant subjects received 480 mL of chocolate milk, buttermilk, whole milk, or skim milk and their breath hydrogen was measured. The breath hydrogen level was the lowest for chocolate milk followed by buttermilk, whole milk, and skim milk. Addition of fibre to milk reduced breath hydrogen by 34-85%. Solomons et al. (1979) also reported reduced rate of breath hydrogen excretion with whole milk as compared to skim milk or lactose solution.

2.7. ROLE OF FERMENTED MILK PRODUCTS IN LACTOSE DIGESTION

2.7.1 Lactose Content of Fermented Milk Products

Fermented milk products such as yogurt, acidophilus milk, and cultured buttermilk generally contain less lactose than the unfermented mix as some of the lactose is fermented to lactic acid by culture organisms. During fermentation of yogurt about 30-40% of lactose is hydrolysed to glucose and galactose (Levitt and Savaiano, 1985; Rao et al., 1985). Goodenough and Kleyn (1976) observed a reduction in lactose content of 34% during yogurt processing due to fermentation to lactic acid. The amount of lactose in yogurt varies. Usually 2-4% non-fat dry milk is added to milk to increase the viscosity of the product (Tamime and Deeth, 1980; Kosikowski, 1982); this is largely responsible for the variation in the lactose contents. Nonfat dry milk, which is usually used for fortification, contains 50-52% lactose (Kosikowski, 1982). Storage time after manufacturing of cultured dairy products also affects lactose levels. Table 2-2 illustrates the lactose content of various dairy products.

Table 2-2. Lactose content of various dairy foods [a]

Food	Lactose (%)
Cow's milk	
Whole milk	4.7
Low-fat milk	4.8 - 5.0
Skim milk	5.0
Chocolate milk	4.1- 4.9
Buttermilk	3.6 - 5.0
Yogurt	
Low fat	1.9 - 7.7
whole milk	4.1 - 4.7
Acidophilus milk	4.4
Kefir, partly skim	4.0
Cheese	
Quarg	3.7 - 5.0
Cottage	trace - 2.9
All others	trace - 1.4
Ice cream (14% cream)	3.6

[a] adapted from Scrimshaw and Murray (1988); Savaiano and Levitt (1987); Jelen and Renz-Schauen (1989).

Alm (1982) measured changes in lactose content during fermentation of buttermilk, kefir, ropy milk, yogurt, acidophilus milk, and bifidus milk. The decrease in lactose content was less in buttermilk, kefir, and ropy milk and was pronounced in yogurt, acidophilus milk, and bifidus milk. The amount of lactose in acidophilus and bifidus milk is similar to that of yogurt (Scrimshaw and Murray, 1988). There is no reduction in lactose content of sweet acidophilus milk as no fermentation takes place (Kim and Gilliland, 1983).

Most ripened cheeses contain trace amounts of lactose as lactose-rich whey is drained from the cheese curd and most of the remaining lactose is fermented during ripening period (Lee and Lillibridge, 1976; Kosikowski, 1982). Camembert and Limburger cheeses contain about 0.4% lactose (Kosikowski, 1982). The lactose content in cottage cheese varies from trace to 2.9% (Welsh, 1976). The amount of lactose in cottage cheese can be further reduced by washing the curd with water during processing. Quarg contains from 3.7 to 5.0% lactose depending on manufacturing method, especially the use of UF which leads to increased lactose content (Jelen and Renz-Schauen, 1989).

2.7.2. Microbial ß-galactosidase Production by Dairy Cultures

Lactic acid bacteria used for processing of cultured dairy products possess two types of the enzyme lactase (ß-galactosidase; E.C. 3.2.1.23). Lactases produced by *Lactobacillus delbrueckii* subsp. *bulgaricus* (formerly, *Lactobacillus bulgaricus*), *Streptococcus salivarius* subsp. *thermophilus* (formerly, *Streptococcus thermophilus*), and *Lactobacillus acidophilus* are called ß-galactoside galactohydrolase (ß-gal) while the lactases possessed by *Lactococcus lactis* subsp. *cremoris* (formerly, *Streptococcus* *cremoris*) and *Lactococcus lactis* subsp. *lactis* (formerly, *Streptococcus lactis*) are called ß-D-phosphogalactoside galactohydrolase (ß-P gal) (Farrow, 1980; Wong et al., 1987; Fisher et al., 1985). These enzymes hydrolyse ß-galactoside linkage of lactose. The organism can ferment lactose by two different pathways. In lactic streptococci, with the exception of *Enterococcus faecium* (formerly, *Streptococcus faecium*) and *S. salivarius* subsp. *thermophilus*, lactose is phosphorylated before hydrolysis to glucose and galactose-6-phosphate.

Farrow (1980) surveyed many strains of lactic acid bacteria for their lactose hydrolysing enzymes and observed that a majority of streptococci contained ß-P gal, except *Enterococcus faecium* and *S. salivarius* subsp. *thermophilus*. Fisher et al. (1985) studied 5 strains of *L. acidophilus* for their lactase activities and reported that all contained 100-fold higher ß-gal as compared to ß-P gal. Several researchers have found that different species of lactobacilli hydrolyse lactose by both ß-gal and ß-P gal enzymes (Permi et al., 1972; Chassy and Thompson, 1983; Fisher et al., 1985).

Lactases are endoenzymes. The whole cell shows very little lactase activity (Jasewicz and Wasserman, 1961). It is necessary to disrupt the cells to release the enzyme for measurable activity. Eaton press and sonication methods are used to rupture the cell wall. Chemicals, such as toluene or acetone, destroy the cell permeability. These treatments release the enzyme activity which is then available for assay purpose (Citti et al., 1965). This results in many fold increase in the measurable enzyme activity (Citti et al., 1965; Kilara and Shahani, 1976). The crude extract has to be dried for further use and purified by removing the cell debris and other interfering proteins.

Most of the lactases are stable to sonication or chemical treatment. However, an increase in temperature caused by the sonication could lead to inactivation of liberated enzymes (Shah, unpublished data). Among 40 strains of *Lactococcus lactis* subsp. *lactis* studied, Wierzbicki and Kosikowski (1973) observed only one strain possessing lactase which was unstable to sonic vibration or toluene treatment.

L. delbrueckii subsp. *bulgaricus* contains higher enzyme activity than *S. salivarius* subsp. *thermophilus* (Wierzbicki and Kosikowski, 1973; Kilara and Shahani, 1976; Fisher et al., 1985). These two organisms are commonly used for yogurt manufacturing. *Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris*, which are used for processing buttermilk, cottage cheese and sour cream, were reported to exhibit negligible or no lactase activity, however, all these studies (Wierzbicki and Kosikowski, 1973; Kilara and Shahani, 1976; Farrow, 1980) have used ortho-nitrophenyl ß-D-galactopyranoside (ONPG) as a substrate to measure the ß-gal activity from *L. lactis* subsp. *cremoris*, which will not estimate ß-P-gal enzyme. *L. acidophilus, S. salivarius* subsp. *thermophilus* and *L. delbrueckii* subsp. *bulgaricus* exhibit maximum activity near neutral pH range and about 55°C (Greenberg and Mahoney, 1982; Chang and Mahoney, 1989; Ramana Rao and Dutta, 1981; Somkuti and Steinberg, 1979; Smart et al., 1985).

2.7.3. Survival of Microorganisms and Microbial ß-galactosidase

The survival of microorganisms or their ß-galactosidase activity in the gastrointestinal tract is still debated. Several reports (Garvie et al., 1984; Goodenough and Kleyn, 1976; Rao et al., 1985) suggest that *L. delbrueckii* subsp. *bulgaricus* and *S. salivarius* subsp. *thermophilus* may survive gastric digestion whereas Gilliland (1985) and Gilliland and Kim (1984) indicated that the organisms do not survive in the intestinal tract but only serve as a source of lactose hydrolysing enzyme. Wong et al. (1987) could not determine the *S. salivarius* subsp. *thermophilus* survival in the gastrointestinal passage but its ß-galactosidase activity survived. As *S. salivarius* subsp. *thermophilus* is not as acid tolerant as *L. delbrueckii* subsp. *bulgaricus*, it is questionable whether this organism would survive the gastric passage conditions (Humphreys and Plunkett, 1969; Acott and Labuza, 1972). Goodenough and Kleyn (1976) have observed that a significant number of yogurt organisms can survive gastric digestion in rats. Kolars et al. (1984) have shown that the enzyme activity after yogurt consumption is not destroyed in the low pH environment of the stomach. It is claimed that after yogurt is consumed, the culture organisms are exposed to bile salts which may alter the permeability of the cells for efficient lactose hydrolysis. In the presence of bile acids, increases in enzyme activities have been observed (Gilliland and Kim, 1984). However, this mechanism of lactose digestion needs to be confirmed in vivo.

Under normal conditions, low pH environment of the stomach could inactivate lactase enzymes (Jackson and Jelen, 1989). However, Martini et al. (1987a) and Savaiano and Levitt (1987) reported that buffering of stomach acid by yogurt aids in protection of microbial cells and their ß-galactosidase from acid inactivation. Incubation of sonicated culture at pH 3.5 or less results in a rapid, complete and irreversible loss of enzyme activity (Martini et al., 1987a).

Lactobacillus acidophilus has been found to survive better than other lactic acid bacteria in low pH conditions (Conway et al., 1987; Goldin and Gorbach, 1984). The sonication time to release enzyme activity is high for this organism (see chapter 4, p. 80) suggesting a rigid cell wall structure which may explain why this organism may be capable of surviving gastric digestion and colonizing the gut. The therapeutic role of *Lactobacillus acidophilus* has been reported by many researchers (Fernandes et al., 1987; Gilliland, 1985; Goldin and Gorbach, 1984). However, this organism seems to play a limited role in improving lactose digestion in lactose intolerant individuals.

2.7.4. Effect of Cultured Dairy Products on Lactose Digestion

Elie Metchnikoff (1908) concluded from his studies that yogurt culture organisms could arrest putrefaction in the intestinal tract and thus might be beneficial to health. Fermented milks have been very popular in those parts of the world where lactose intolerance is prevalent. Among the fermented milks, yogurt appears to be well tolerated by lactase deficient persons (Alm, 1982; Kolars et al., 1984; Kelly, 1984; Levitt and Savaiano, 1985; Savaiano et al., 1984). Baer (1970) suggested that better tolerance of yogurt is due to reduction in lactose content during fermentation. However, yogurt may contain higher lactose than milk as milk is fortified with milk solids before fermentation (Tamime and Deeth, 1980). Thus it appears that lower lactose content may not be responsible for better tolerance of yogurt.

Sweet acidophilus milk is made by adding high concentrations of *Lactobacillus acidophilus* cells to milk (Ayebo et al., 1980). Kim and Gilliland (1983) showed that lactose malabsorbers are able to tolerate acidophilus milk whereas several reports have claimed that acidophilus milk is not beneficial to lactose intolerant individuals (Payne et al., 1981; Reasoner et al., 1981; Welsh, 1981; Newcomer et al., 1983; Onwulata et al., 1989). McDonough et al. (1987) also observed that sweet acidophilus milk containing viable organisms did not enhance lactose digestion. However, sweet acidophilus milk with sonicated cells was found to be beneficial for lactose malabsorbers (Wong et al., 1987).

Gallagher et al. (1974) investigated the lactose malabsorption effects of yogurt, cottage cheese, cultured buttermilk, and milk with three lactose intolerant subjects. The subjects tolerated yogurt, cultured buttermilk and cottage cheese better than milk.

Alm (1982) fed 500 mL of yogurt, acidophilus milk, and low fat milk to 12 lactose malabsorbers. The total lactose content in dairy products was 24.6, 18.1, and

11.4 g in low fat milk, acidophilus milk, and yogurt, respectively. Blood glucose was measured and lactose malabsorption symptoms were noted following the consumption. The subjects tolerated yogurt and acidophilus milk well whereas consumption of milk resulted in symptoms of gastrointestinal distress. The improved tolerance of yogurt or acidophilus milk in this study could have been due to lower lactose in yogurt and acidophilus milk as compared to milk.

Gilliland and Kim (1984) observed the effects of viable yogurt organisms on lactose digestion in lactase deficient humans. Yogurt, pasteurized yogurt, and acidified yogurt mix containing 4.3, 4.3 and 6.26% lactose were fed to 6 lactose intolerant subjects. Breath hydrogen level was the lowest for yogurt and the highest for acidified yogurt mix. Lactose malabsorption symptoms after feeding experimental diets were not reported in this study. Better tolerance of yogurt as compared to that of the acidified yogurt mix probably was due to lower lactose content of yogurt.

In a study by Kolars et al. (1984), ten lactose intolerant individuals received experimental diets including milk, lactose solution, lactulose and yogurt. Breath hydrogen test was used to measure the malabsorption of lactose and the symptoms of lactose intolerance were also recorded. The results showed that yogurt produced the least amount of hydrogen in the lactase deficient subjects.

Savaiano et al. (1984) studied the effects of milk, yogurt, pasteurized yogurt, cultured buttermilk and sweet acidophilus milk on absorption of lactose in lactose malabsorbers. Each of the four products containing 20 g of lactose was fed to 9 lactose malabsorbers. Breath hydrogen test was used to measure the absorption of lactose. Yogurt produced the lowest breath hydrogen increase from the base level and sweet acidophilus milk the highest. Eight of the nine subjects experienced gastrointestinal problems with cultured buttermilk but none with pasteurized yogurt. Slower rate of gastric emptying may have been responsible for efficient hydrolysis of lactose by residual lactase enzyme of the small intestine from pasteurized yogurt as pasteurized yogurt contained neither viable microorganisms nor enzyme activity.

Improved lactose digestion from yogurt has been confirmed by several other reports (Martini et al., 1987a; Martini et al., 1987b; McDonough et al., 1987; Martini and Savaiano, 1988; Onwulata et al., 1989).

In a recent study by Onwulata et al. (1989) yogurt, sweet acidophilus milk, whole milk, whole milk with lactase tablet, and lactose hydrolysed milk were fed to 10 lactase deficient black subjects. Each product contained 18 g lactose except lactose hydrolysed milk which contained only 5 g lactose. Breath hydrogen was measured to monitor lactose malabsorption. Sweet acidophilus milk was ineffective in alleviating the symptoms of lactose intolerance. Microbial lactase activity in yogurt organisms was found to be superior to a lactase enzyme preparation in alleviating lactose intolerance problems, as the free lactase was probably deactivated by the low pH in the stomach.

Wytock and DiPalma (1988), in their study, found that two of the three brands of yogurt improved lactose digestion as measured by breath hydrogen whereas the third brand produced symptoms of gastrointestinal discomfort. Schaafsma et al. (1988) reported that yogurt microbial lactase did not contribute significantly to the digestion of lactose in rats. The animals were fed diets including milk, lactase-treated milk, yogurt and pasteurized yogurt. Yogurt or pasteurized yogurt did not show any difference in feed efficiency, blood galactose response and relative caecum weight indicating that yogurt culture organisms or their lactase activities were not necessary for efficient digestion and absorption of lactose.

The exogenous enzyme activity from yogurt microbial cultures or free added enzyme would be inactivated by low pH of stomach. Irreversible inactivation of the neutral lactases in acidic pH condition is known to occur (Savaiano et al., 1984; Jackson and Jelen, 1989). Thus it appears that the exogenous lactase activity of microbial cultures may not be responsible for efficient hydrolysis of lactose in lactase deficient persons. A study with lactose intolerant subjects showed that pasteurized yogurt can be well tolerated (Savaiano et al., 1984). The tolerance of pasteurized yogurt containing neither viable organisms nor ß-galactosidase activity suggests that there may be other factors contributing to efficient lactose digestion.

2.7.5. Alternate Mechanisms for Lactose Digestion and Absorption

Gastric emptying and intestinal transit time depend on the composition of food. Milk consumed with a meal could result in delayed gastric emptying leading to slower delivery of lactose to the intestine where it can be hydrolysed by the residual ß-galactosidase in the small intestine as shown by Solomons et al. (1985b) and Martini and Savaiano (1988). In these studies, consumption of milk with a meal was found to reduce the lactose intolerance symptoms, presumably due to delayed gastric emptying and/ or the dilution effect of the meal in the gut. In the study of Martini and Savaiano (1988), three subjects from the total of 12 exhibited symptoms of discomfort following consumption of skim milk with a meal as compared to 8 subjects from 12 with skim milk only. Solomons et al. (1985a) studied 13 lactose intolerant subjects who received 360 mL milk with breakfast. The meal reduced the amount of lactose reaching the colon during 6 hr following ingestion by 47%. Welsh and Hall (1977) reported slower gastric emptying of chocolate milk than milk. Consumption of chocolate milk reduced the symptoms of lactose malabsorption as compared to unflavored milk. Nguyen et al. (1982) showed that the addition of fibre to milk reduced breath hydrogen by 34-85% and symptoms of lactose malabsorption were also reduced. Thus it appears that food consistency is likely to be an important factor responsible for efficient digestion and absorption of lactose in lactase deficient individuals.

2.8. QUARG

2.8.1. Definition

Quarg is an acid coagulated casein paste obtained from lactic acid fermentation using buttermilk or sour-cream cultures. It is classified as a soft, unripened, fresh cheese and is generally produced from skim milk (Mann, 1978). West Germany is one of the highest quarg consuming countries. The minimum requirement for total solids in quarg is 16% in Denmark while in Germany it is 18% (Jelen and Renz-Schauen, 1989). At the present time, there is no minimum limit for protein content. Some of the quarg made by UF may contain up to 5% lactose (Table 2-2).

2.8.2. Technology

The basic technology of quarg processing is outlined by Kroger (1980). Traditionally, quarg is prepared by fermentation of milk with mesophilic cultures. Milk is usually coagulated at about 20-21°C; however, higher temperature (25-30°C) coagulation has also been practised. A small amount of rennet is added for stabilization of protein and for preventing losses of casein fines (Jelen and Renz-Schauen, 1989). After coagulation of the milk, removal of whey is accomplished by cutting the gel into cubes and draining with cheese cloth. However, for processing of large amounts of quarg, this type of whey removal is too laborious. Westfalia quarg separator has been used for more efficient removal of whey in larger processing plants. Modern methods of quarg manufacture are highly automated and controlled.

The whey obtained from conventional method of pasteurized skim milk quarg processing contains about 0.7% whey protein (Sheth et al., 1988; Jelen and Renz-Schauen, 1989). Some of this whey protein can be recovered by precipitating the heat coagulable protein from milk using high-heat treatment which would also improve the yield of quarg. All new technologies in quarg processing are geared towards recovery of whey proteins by high heat-treatment of skim milk or whey (Lang, 1980; Kroger, 1980).

Lang (1980) has described several methods of quarg manufacture aimed to recover whey proteins. A new method of quarg manufacture called "thermo-process" has been reported by Lang (1980) where skim milk is heated at high temperature (95-98°C) for 2.5 min to denature whey proteins followed by culturing and renneting. The milk is then incubated, usually overnight. Additional heat treatment is given to the fermented milk when the pH decreases to 4.5. The advantage of additional heat treatment is that the precipitated whey proteins are retained in the quarg. The fermented milk is cooled and whey is separated in a Westfalia quarg separator.

In the "centri-whey" process of protein precipitation, whey is heated to about 96°C and held for 20 min to precipitate whey protein. The whey is then passed through a separator to obtain a liquid protein concentrate of 15-18% total solids which is added to milk for processing of quarg.

Puhan and Gallman (1980) describe an ultrafiltration (UF) method for quarg manufacture. UF can retain all whey proteins. In this method, pasteurized skim milk is inoculated with mesophilic cultures and incubated at 23°C till the pH decreases to 5.7-5.9. The milk is then concentrated by UF and the fermentation is continued to pH 4.5. Several other UF methods are now commonly used by German quarg processors (Friis, 1981; Jelen and Renz-Schauen, 1989).

Lipatov et al. (1978) studied the effects of temperature on the production of quarg and reported that coagulation temperature of milk above 38°C is likely to affect cheese structure as there is shrinkage of curd and syneresis. The body of quarg can be coarse and rubbery. Manus (1973) and Babella and Szabo (1974) used yogurt culture (*Streptococcus salivarius* subsp. *thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*) for quarg processing instead of traditional mesophilic culture. By heating the curd at different temperatures and times, they could improve sensory quality of quarg. Quarg processing using *Lactobacillus acidophilus* cultures has not been reported in the literature.

2.9. USE OF WHEY IN HUMAN FOODS

In the past, cheese manufacturers were forced to dispose of whey as no suitable or economical processes were available for recovery of its components. Recent developments in processing such as ultrafiltration (UF), reverse osmosis (RO), and demineralization have allowed whey to become a versatile by-product for the food industry. Components of whey can be modified by these and other methods and proteins and minerals can be extracted.

Whole dried whey is prepared first by condensing the whey by evaporation and then spray drying to powder. Dried whey is used in a variety of food such as ice cream, sherbet, baked goods, confectioneries, meat products, etc. Heat denaturation of whey proteins or UF can be used to prepare whey protein concentrates for food use. Use of whey solids in infant formulations has been studied extensively by Mathur and Shahani (1979). Whey is found to be useful as a base for producing soups and gravies (Shahani et al., 1978). Some uses of whey are presented in Table 2-3.

Whey is rich in vitamins especially those of B group which are limiting in

Table 2-3. Some uses of whey in human foods [a]

Beverages	Fruit juices Vegetable juices (e.g. tomato) Soups (e.g. cream of tomato)
Bakery	Cake, Cookies, Crackers Sour dough and Rye bread Breading mix for fried foods
Meat products	Meat loaves and spreads Sausage and Frankfurters
Dairy products	Ice cream, Sherbet, Ice cream coatings Process cheese, and Cheese spreads Yogurt Modified milks
Confections	Fudge, Caramel, Chocolate
Whey cheeses	Ricotta, Mysost

[a] adapted from Webb (1972); Kosikowski (1979); Devkota (1990); Jelen (1990).

flours, thus can be used with flours for bread making. UF and RO have been applied for the manufacture of undenatured whey protein concentrate (WPC). Whey can be demineralized by ion exchange or electrodialysis prior to spray drying to produce dry demineralized whey for whey protein based infant formulas (Hayes, 1985). Dried WPC is a food ingredient of both functional and nutritional values. WPC has wide range of use in food ingredients.

Demineralized whey can be treated with lactase enzyme to produce protein rich sweet syrup. Hydrolysed lactose syrups can be used in ice cream and confectionery. Utilization of cottage cheese whey in processed cheese-like dairy spreads has been reported by Jelen and Yehya (1981). Also, whey has been used successfully in whey based beverages (Holsinger et al., 1974; Dodds, 1989; Prendergast, 1985).

Whey can also be used in yogurt and other fermented dairy products. Hartman (1975) prepared yogurt formulations containing neutralized (pH 6.55) liquid cottage cheese whey or sweet whey with various modified dry wheys and reported that sweet whey solids or neutralized cottage cheese whey solids can be used in yogurt at the rate of 1-2% without any adverse effect. Prodanski (1970) made yogurt from milk fortified with proteins which had been recovered from whey and buttermilk. The addition of whey or buttermilk proteins resulted in good consistency and flavor. Utilization of acid whey in frozen yogurt was reported by Hekmati and Bradley (1979). They used about 44% fluid whey which resulted in improved body and texture. The characteristics of dry and acid cottage cheese whey offer special opportunity for application in cultured products with high acidity such as sour cream, cheese and other flavored dips, dairy spreads, and fruit yogurts (Nielsen, 1976).

In Nepal, cheese plants use yak and buffalo milk to manufacture several types of cheese and the whey produced is mainly of the sweet type. "Chhurpi", a whey cheese similar to ricotta, is popular in some parts of the hilly region of the country. Chhurpi is processed by heat- coagulating proteins from whey or buttermilk followed by sun drying.

Most of the cheese factories are located in hilly zones of Nepal where means of transportation is limited. Cheeses are carried to urban areas by porters. It may not be feasible or economical to transport whey as it contains about 93% water. High amount of water in whey and high costs of energy required to evaporate the water from the whey would make it difficult for whey to be evaporated or dried. Rice and cornmeal, which are traditionally cooked in water, can be cooked in whey thus utilizing the whey nutrients. It will be most practical to use the whey by the people living in the vicinity of the cheese factories if lactose from the whey would be tolerated.

Milk is in short supply in Nepal. Dairy Development Corporation of Nepal has been able to supply about 50% of the demand of the urban consumers. The rest of the demand is fulfilled by importing milk powder to supplement local collection to fulfill the heavy demand. The possibilities of using nonfat dry milk and dry whey for processing of yogurt have been studied earlier (Shah, 1982). Dry whey is much cheaper than milk powder. Hence, use of dry whey as an ingredient in yogurt or other dairy products would afford economic advantage. Attempts should be made to use surplus whey from around the world for nutritional fortification in developing countries like Nepal. This will also reduce the importation of milk powder. However, use of whey in foods could be difficult in countries with high incidence of lactose intolerance.

An attempt has been made in this project to ascertain the survival of the microorganisms and their ß-galactosidases under acidic conditions, to test if foods containing higher solids would result in better tolerance of lactose, and to propose a suitable way to use whey in foods for consumption by lactose intolerant populations.

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3. INDICATION OF THE MAGNITUDE OF LACTOSE INTOLERANCE PROBLEM IN DIFFERENT ETHNIC GROUPS OF NEPAL[1]

3.1. INTRODUCTION

The ingestion of milk causes abdominal cramps, bloating, flatulence and diarrhea in some children and adults (1). Many of these individuals can be classified as lactose malabsorbers as their bodies do not produce sufficient lactase (beta-galactosidase, E.C. 3.2.1.23) enzyme in the intestine to hydrolyse lactose, the milk sugar. As a result, unhydrolysed lactose is not absorbed and it moves through large intestine where osmotic effects and fermentation by gut bacteria often produce gastrointestinal discomfort, diarrhea, etc. (2, 3, 4, 5). Studies have demonstrated a widespread prevalence of this disorder among Asians, Africans and American Negroes in contrast to a low prevalence in Caucasians (5, 6, 7, 8, 9, 10). The incidence of lactose intolerance has been reported to be 97-98% among Thais, 90% among Chinese, 67% among Indians, 70% among American Negroes, 78% among African Negroes, but only 10-15% among U.S. Whites and 3-4% among Scandinavian populations (4, 6, 9). Approximately 80% of non-Caucasians are reported to be lactose intolerant (5). Rosensweig (9) has reported an incidence of lactase deficiency to be 95% for Asians.

The high incidence of lactose intolerance among Asians and Africans could be attributed to low milk production and consumption in these areas in contrast to high milk production in the areas where Caucasians live (9). Simoons, as mentioned by

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Hourigan and Mittal (11) has also observed high incidence of lactose intolerance in the traditional non-dairying areas. Lactase deficiency in areas of low milk consumption may have resulted due to an adaptive decline in the enzyme concentration following milk withdrawal as the child grows older (1, 12). However, in certain areas of Asia and Africa with traditional milk production, lactose intolerance is not a problem (6, 10, 11).

In view of these observations it becomes important to assess the prevalence of lactose intolerance before a new product, with a substantial lactose content, is introduced in those areas of the world where lactose intolerance could pose a problem. There is no study reported from Nepal regarding the incidence of lactose intolerance. The recent emergence of cheese manufacturing as an increasingly important industrial activity in Nepal could lead to similar problems as in other cheese producing countries regarding whey disposal. However, in a country with severe malnutrition such as Nepal, the whey should be utilized as a valuable human nutrient source, if its lactose content could be tolerated. The purpose of this study was to conduct a survey of several ethnic groups typical of the Nepalese population with the objective of estimating the magnitude of the lactose intolerance problem in this country.

3.2. MATERIALS AND METHODS

3.2.1. Background

There are 4 major castes of people in Nepal - Brahmin, Chhetri, Baishya, and Shudra. Brahmin and Chhetri are classified as upper caste people whereas Baishya (including Newar, Gurung, Tharu, and other groups) and Shudra, as well as other ethnic groups outside the caste system are generally considered as lower caste people.

Newar people are the indigenous inhabitants of the valley of Kathmandu. Gurungs and Tharus are the aboriginal inhabitants of the hilly and plain regions of the country. There are some cultural differences among these groups, including the differences in milk usage patterns. Aryan descendents, such as Brahmins and Chhetris, are traditional dairying people. Tharus are non-dairying people; although they keep animals, they do not milk them. Milk drinking in other ethnic groups, such as Newar and Gurung, depends on availability and socioeconomic status.

This study was conducted in the Chitwan district of Nepal. The kingdom of Nepal is divided into 14 zones with 75 districts, consisting of several village panchayats. A ward is the smallest unit of a village panchayat and 11 wards make up a village panchayat. Six village panchayats included in this study were: Bharatpur (ward numbers 5, 6), Mangalpur (ward number 1), Shardanagar (ward numbers 1, 2, 3, 6 and 9), Dibyanagar (ward number 6), Gunjnagar (ward numbers 1, 2, 4, 5, 6, and 9), and Meghauli (ward number 8). Five ethnic groups included in this group were Brahmin, Chhetri, Newar, Gurung, and Tharu.

3.2.2. Survey Methodology

The lists of households were obtained from the respective Pradhan panch (elected chief of a village panchayat). Fifty households from each ethnic group were randomly selected from different ward by drawing lots.

A survey questionnaire was developed which consisted of biographical information, milk production and consumption habits and a recall indication of symptoms after milk ingestion. In each case, the representative member of the family was contacted and informed of the nature of the proposed study. Then the questionnaire was completed by the family head for the whole family or by surveyor in case the family head was illiterate.

3.2.3. Data Analysis

Results from all the households that reported milk-drinking habits were tabulated and the average values for milk consumption in different ethnic groups were subjected to analysis of variance for determination of statistical significance. Duncan's New Multiple-Range Test was used to establish the difference in means of milk consumption for the 5 ethnic groups as described by Steel and Torrie (13). Correlation between milk production and consumption data versus reported lactose intolerance symptoms was determined using Shazam program of the University of Alberta computing services.

3.3. RESULTS AND DISCUSSION

The results of the lactose intolerance survey in the 5 Nepali ethnic groups studied are represented in Table 3-1. The detection of intolerance problem was subjectively based on a family history of symptoms such as abdominal cramps, bloating and diarrhea noted after milk ingestion. Data for only those families where milk drinking was customary are included in Table 3-1. The highest number of milk users were among Brahmins and lowest number of milk users among Tharus.

About 69% of the Tharu households reporting milk consumption indicated lactose intolerance problems, followed by 34% in Newars, 22% in Gurungs, 17% in Brahmins, and 13% in Chhetris. In the whole population studied, the highest lactose intolerance problem was recorded in Tharus. About 19% of all individuals included in the survey indicated lactose intolerance problem in Tharu group as compared with 7% or less in other ethnic groups.

Table 3-2 illustrates average milk production and consumption as reported by the different ethnic groups. On the average the recorded milk consumption per head per day was very low in all the ethnic groups. Although milk consumption data were the highest in the Brahmin group, there was no statistically significant difference (P>.05) among Brahmin, Chhetri, Newar and Gurung groups. However, milk consumption was significantly lower (P<.05) in Tharu group. There was a significant (P<.05) negative correlation (r= -0.7) between milk consumption and lactose intolerance in different ethnic groups. This might suggest that the incidence of lactose intolerance could be less severe if milk consumption habits would be increased.

Table 3-3 shows the number of illiterate persons in different ethnic groups. According to the World Almanac (14) the literacy rate of Nepal is 23%. The results obtained in this study showed higher literacy rate. This discrepancy may be due to the fact that Chitwan is one of the most developed and prosperous districts in Nepal. The reported literacy rate in the Tharu group was 48% as compared with 22% or less in other ethnic groups. The rate of lactose intolerance in Tharu population was also very high as compared with other ethnic groups. This suggests a possible correlation between the levels of lactose intolerance and illiteracy rate in Tharu population. Milk drinking after weaning is very uncommon in most of the Tharu population. Because of the lack of education, the Tharus may not be aware of the nutritional attributes of milk.

Table 3-4 lists the types and frequency of discomfort indicators reported by the intolerant subjects. Abdominal cramps and abdominal cramps/diarrhea after milk ingestion were the most frequent symptoms. About 12% of the subjects complained of diarrhea and 6% of bloat after milk ingestion.

Seven out of the 69 persons indicating lactose intolerance problems claimed that ingestion of dahi (a fermented milk product similar to yogurt) did not cause any discomfort. In Nepal, indigenous home-made dahi is popular and is used as part of the diet. Studies have shown that yogurt can be well-tolerated by lactose intolerant individuals (15, 16, 17).

3.4. CONCLUSIONS

The prevalence of lactose intolerance in this study was based on a family survey of different ethnic groups typical of Nepal. High incidence of lactose intolerance recorded in Tharu population appeared to be correlated with low milk consumption habits and possibly with the lack of education since the illiteracy rate was very high in this group. Overall low incidence of lactose intolerance recorded in this study as compared to reports for other Asian populations may be due to the universally low level of milk consumed by all ethnic groups as this probably was insufficient to show clinical symptoms of lactase deficiency noticeable to many of the subjects interviewed. Milk intolerance should be anticipated also in the households where milk was not being used. On an average the milk consumption was 353 mL or less in all the ethnic groups. This means that the indication of acute lactose intolerance symptoms could be much higher than observed in this study if a proper lactose intolerance test would be administered. The test dose commonly used is 50 g of lactose which is the amount found in approximately one litre of milk (5, 12). Lactase deficiency had not become clinically significant to the subjects in this study, since most of the population drank very little milk. Further work is needed to assess the exact level of the lactose intolerance problem in the many diverse ethnic groups living in Nepal by administering the proper lactose intolerance test. The incidence of lactose intolerance should be known prior to introduction of a new product containing substantial amount of lactose to populations that might be expected to be lactase deficient.

3.5. ACKNOWLEDGEMENTS

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3.6. TABLES AND FIGURES

Table 3-1. Prevalence of lactose intolerance in different ethnic groups

Ethnic group	Number of households		Number of lactose intolerant persons			Total population in the survey
	using milk[a]	with lactose intolerance problem	Adults	Children	Total	
Brahmin	47	8(17.0%)	1	11	12	318
Chhetri	40	5(12.5%)	1	6	7	232
Newar	41	14(34.2%)	12	6	18	263
Gurung	41	9(21.9%)	3	13	16	228
Tharu	16	11(68.7%)	4	12	16	86

[a] 50 households interviewed for each ethnic group

Table 3-2. Milk production and consumption patterns in different ethnic groups

Ethnic group	Average milk production per household (mL)	Average milk consumption per head per day (mL)
Brahmin	7800	353
Chhetri	4338	306
Newar	4000	313
Gurung	2864	289
Tharu	1063	171[a]

[a] Significantly different ($P< 0.05$) from the rest of the data in the column

Table 3-3. Illiteracy rate in different ethnic groups as ascertained in this survey

Ethnic groups	Illiteracy (%)
Brahmin	20
Chhetri	20
Newar	22
Gurung	21
Tharu	48

Table 3-4. Types and frequency of discomfort reported among lactose intolerant subjects

Types of discomfort after drinking milk	Number of cases	Percent of total cases
Stomach cramps	27	39.1
Stomach cramps/diarrhea	30	43.5
Diarrhea	8	11.6
Bloat	4	5.8
Total	69	100

3.7. REFERENCES

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4. SURVIVAL OF LACTIC ACID BACTERIA AND THEIR LACTASES UNDER ACIDIC CONDITIONS[1]

4.1. INTRODUCTION

The Russian Nobel laureate, Metchnikoff (1908), first suggested that consumption of fermented milk with *Lactobacillus acidophilus* bacteria was beneficial for suppressing the growth of putrefactive bacteria and for prolonging life. The use of fermented dairy products is common in the areas of the world where lactose malabsorption is prevalent (Gallagher et al., 1974; Kretchmer, 1972). The therapeutic potential of yogurt microorganisms for preventing intestinal upsets has been advocated (Goodenough and Kleyn, 1976; Speck, 1977). Yogurt has been found to be well tolerated by lactase deficient subjects (Gallagher et al., 1974; Gilliland and Kim, 1984; Kolars et al., 1984). The beneficial effect of yogurt is claimed to be dependent on the ingestion of viable yogurt bacteria (Savaiano et al., 1984), however, the ability of microorganisms to survive within the gastrointestinal tract and to hydrolyze lactose through action of their ß-galactosidase (ß-gal) enzyme is still debated (Renner, 1989, working paper of Group F-20, Int. Dairy Fed., Brussels, Belgium).

The survival of microorganisms is affected by low pH of the environment. Hood and Zottola (1988) observed that the *L. acidophilus* populations decreased rapidly at pH 2.0, however, there was no decrease in the number of viable cells at pH 4.0. As *Streptococcus salivarius* subsp. *thermophilus* (formerly, *Streptococcus*

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thermophilus) is not as acid tolerant as *L. delbruekii* subsp. *bulgaricus* (formerly, *L. bulgaricus*) it is questionable whether this organism would survive the gastric passage conditions (Humphreys and Plunkett, 1969; Acott and Labuza, 1972). Wong et al. (1987) could not demonstrate the *S. salivarius* subsp. *thermophilus* survival in the gastrointestinal passage, but its ß-galactosidase survived and contributed to improved lactose digestion. Goodenough and Kleyn (1976) have shown that a significant number of yogurt organisms can survive passage through the gastrointestinal tract. Several reports indicated that the lactase activity from yogurt cultures is not destroyed in the acid environment of the stomach and can enhance intestinal absorption of lactose (Gilliland and Kim, 1984; Savaiano et al., 1984; Kolars et al., 1984; Martini et al., 1987; Savaiano and Levitt, 1987).

Lactococcus lactis subsp. *cremoris* (formerly, *Streptococcus cremoris*) and *Lactococcus lactis* subsp. *lactis* (formerly, *Streptococcus lactis*) were claimed to exhibit negligible extracellular ß-gal activity (Kilara and Shahani, 1976; Farrow, 1980), while the ß-gal activities of *Lactobacillus helveticus*, *L. delbruekii* subsp. *bulgaricus*, and *S. salivarius* subsp. *thermophilus* are the highest among the lactic acid bacteria (Wierzbicki and Kosikowski, 1973). Lactase activity of *L. acidophilus* has not been reported in so far as could be ascertained.

Lactase is an endoenzyme, and whole microbial cells exhibit very little exogenous lactase activity (Jasewicz and Wasserman, 1961). Cell lysis by sonication or Eaton press method has been shown to increase the ß-gal activity several times (Kilara and Shahani, 1976; Kolars et al., 1984). The ß-gal enzyme in *S. salivarius* subsp. *thermophilus* is cytosolic and exhibits maximal activity near neutral pH and at optimum temperature of 55-57^{o}C (Somkuti and Steinberg, 1979; Greenberg and Mahoney, 1982).

The objectives of this study were: (1) to compare the ß-galactosidase activities of *L. delbruekii* subsp. *bulgaricus, S. salivarius* subsp. *thermophilus, L. acidophilus,* and *L. lactis* subsp. *cremoris* in skim milk and broth systems before and after sonication, (2) to determine lactase activity of 2 specific strains of *L. lactis* subsp. *cremoris*, before and after sonication, using o-nitrophenyl ß-D-galactopyranoside-6 phosphate (ONPG-6P) as a more appropriate substrate, in comparison to two specific strains of *L. delbrueckii* subsp. *bulgaricus*, (3) to study the properties of crude ß-galactosidases isolated from unspecified strains of *L. delbruekii* subsp. *bulgaricus, S. salivarius* subsp. *thermophilus, L. acidophilus,* and *L. lactis* subsp. *cremoris* as well as from *L. delbrueckii* subsp. *bulgaricus* 11842, and (4) to determine the effect of acidic conditions on survival of the intact microorganisms and of their ß-galactosidases before and after sonication.

4.2. MATERIALS AND METHODS

4.2.1. Propagation of Cultures

Pure cultures of *S. salivarius* subsp. *thermophilus* and *L. delbruekii* subsp. *bulgaricus* were isolated from a commercial yogurt sample. The isolated cultures were examined for purity by conventional methods (Hardie et al., 1986). Freeze-dried culture of *L. acidophilus* was obtained from Department of Microbiology, North Carolina State University, and a culture of *L. lactis* subsp. *cremoris* was provided by Dr. M. Stiles of the Department of Food Science, University of Alberta.

Each culture was maintained in sterile 12% (w/v) reconstituted nonfat dry milk (NDM) as well as Difco All-purpose Tween (APT) broth. Sterile 100 mL batches of NDM or APT broth were inoculated with 1% of each culture and incubated at 43°C for

L. delbruekii subsp. *bulgaricus*, 37°C for *S. salivarius* subsp. *thermophilus* and *L. acidophilus* and 31°C for *L. lactis* subsp. *cremoris*. These incubation temperatures were used throughout this study, unless indicated otherwise. Cultures were transferred successively at least three times before use.

In a separate follow-up study, freeze dried cultures of two strains of *L. delbrueckii* subsp. *bulgaricus* (11842 and 7994), and two strains of *Lactococcus lactis* subsp. *cremoris* (14365 and 9596), were obtained from ATCC (American Type Culture Collection, Rockville, MD). According to the recommendations supplied by ATCC, *L. delbrueckii* subsp. *bulgaricus* 11842 was maintained in sterile 12% non-fat dry milk (NDM), and all the other three bacterial strains were maintained in sterile tomato juice-yeast extract milk (TYM) media. *L. delbrueckii* subsp. *bulgaricus* 11842 was grown at 45°C, *L. delbrueckii* subsp. *bulgaricus* 7994 at 37°C, and the two *Lactococci* were grown at 26°C, for 18 hour. The cultures were transferred successively three times before use.

4.2.2. Effect of Sonication on Release of ß-galactosidase

Ten g of each culture were mixed with distilled water, blended for 1 min and the final volume was made to 100 mL in a volumetric flask. Aliquots of the diluted sample held in an ice-bath were sonicated for 20 min for *L. acidophilus* culture and for 10 min for other cultures using Sonic 300 dismembrator (Artek Systems Corp., Farmindale, N Y 11735) at a frequency of 16 KHz. Samples were taken every min and 1 mL portions of the sonicated solution were used to determine enzyme activity. The temperature of the sample was also checked every minute to avoid a rise in temperature during sonication. In this arrangement, the sample temperature during sonication did not exceed 20°C.

4.2.3. Assay for ß-galactosidase

To determine the enzyme activity, 10 g of each sonicated or unsonicated culture were mixed with distilled water, blended for 1 min and the final volume was made to 100 mL in a volumetric flask. One mL of the solution was used in the assay, carried out according to Citti et al. (1965). Solutions of 0.005 M o-nitrophenyl-ß-D-galactopyranoside (ONPG) or o-nitrophenyl-ß-D-galactopyranoside-6 phosphate (ONPG-6P) were prepared in 0.1 M phosphate buffer, pH 7.0, and 1 mL aliquots of the diluted samples were incubated with 5 mL ONPG or ONPG-6P solution for 15 min at 37°C. The reaction was stopped by adding 2.5 mL 1 M cold sodium carbonate. The amount of o-nitrophenol (ONP) released was measured with a spectronic 21 spectrophotometer (Bausch and Lomb Inc., Rochester, N. Y.) at 420 nm. The unit of lactase activity was estimated according to the method of Mahoney et al. (1975) as the amount of the enzyme which liberated one µmole o-nitrophenol from ONPG or ONPG-6P per min per gram sample at 37°C. Chemicals were obtained from Sigma (P. O. Box 14508, St. Louis, MO 63178).

4.2.4. Production of Lactase in Broth Systems

The organisms grown in the APT broth were routinely propagated and transferred successively 3 times, then the active cultures were transferred to Lactobacilli MRS broth (Difco Laboratories, Detroit, Michigan) or Difco APT broth containing either 0.01 g/mL glucose (GAPT) or 0.01 g/mL lactose (LAPT). The cultures were grown for 18 h. At the end of the incubation period, cultures were immediately chilled and centrifuged at 16300 x g for 10 min at 1°C in a Sorvall Model RC-5B (Du Pont Co., Diagnostic and Bioresearch systems, Wilmington, Delaware 19898) superspeed centrifuge. The harvested cells were washed by dissolving in 100 mL distilled water, recentrifuged, and suspended in 20 mL distilled water for sonication in two portions. One portion was used for the ß-gal assay which represented the total enzyme activity. The other portion was centrifuged at 13100 x g for 10 min to remove the cell debris. The supernatant liquid was also assayed for ß-gal to estimate the free enzyme which was not bound to the cell wall. The difference between the two assays represented the enzyme bound to the cell wall. The cell debris were dried at 105^{o}C for 2.5 h to obtain the dry weight of cell suspensions.

4.2.5. Preparation of the crude lactase from the microorganisms

The method of Jasewicz and Wasserman (1961) was used with minor modifications. Harvesting of the cell from the broth was as described above. The harvested cells were dissolved in 20 mL distilled water and the cell suspension was mixed vigorously with 100 mL cold acetone. The treated cells were placed in an ice bath with occasional shaking for 15 min and then filtered through Whatman number 3 filter paper. The crude enzyme was air dried on the filter paper, refrigerated in a desiccator, and assayed within a few days.

4.2.6. Properties of ß-galactosidase

To determine the optimum pH and temperature conditions of the crude enzyme preparations, the enzyme isolated from acetone precipitation was diluted 17000 times in distilled water for *L. delbrueckii* subsp. *bulgaricus* 11842, 1500 times in the case of the unspecified strains of *L. delbrueckii* subsp. *bulgaricus* and *S. salivarius* subsp. *thermophilus* lactases, and 250 times for *L. acidophilus* lactase. The optimum pH of the enzyme was determined by measuring enzyme activity in phosphate buffer at 37^{o}C over a pH range of 4.5-7.5 (8.5 in case of *S. salivarius* subsp. *thermophilus* lactase). Different proportions of 0.2 M mono- and disodium phosphate buffer were used to obtain the desired pH. The optimum temperature for enzyme activity was then determined by measuring enzyme activity at the optimum pH over a temperature range of 35-65°C.

4.2.7. Effect of Acidic Conditions on Survival of Microorganisms and their Lactases

To evaluate survival of the four unspecified bacterial cultures under acidic conditions, aliquots of active cultures grown in NDM were adjusted to pH 3.5, 2.5, or 1.5 with 1 N hydrochloric acid and incubated at 37°C for 2 h. Samples were taken at 30 min intervals and the viable organisms were enumerated by plate counts of all the samples using a 10-fold serial dilutions prepared with 0.1 M phosphate buffer, pH 7.0. Difco-Lactobacilli MRS agar was used for enumeration of *L. delbruekii* subsp. *bulgaricus*, and *L. acidophilus* and Difco-APT agar for *S. salivarius* subsp. *thermophilus* and *L. lactis* subsp. *cremoris*. Duplicate plates were incubated anaerobically using BBL anaerobic jars (BBL Microbiology Systems, Division of Becton, Dickinson and Co., Cockeysville, MD 21030) for either 72 h at 37°C for *L. acidophilus*, *L. delbruekii* subsp. *bulgaricus*, and *S. salivarius* subsp. *thermophilus*, or for 48 h at 31°C for *L. lactis* subsp. *cremoris*. To study the effect of acidic conditions on the loss of activity of the free or cellular lactases, 25 mL aliquots of active cultures (both unsonicated and sonicated) were adjusted to pH 3.5, 2.5, and 1.5 with 1 N hydrochloric acid and incubated at 37°C for 3 h. Hourly samples were taken and assayed for ß-gal activity using ONPG substrate as described above.

Unless otherwise indicated, all the experiments and analyses were repeated at least twice. The results shown are the averages of all available data.

4.3. RESULTS AND DISCUSSION

4.3.1. Effect of Sonication on Release of ß-galactosidase

The effect of sonication at 16 KHz on the four different bacterial cultures is shown in Fig 4-1 and similar results with two specific strains each of *Lactobacilli* and *Lactococci* are illustrated in Fig 4-2. Upon sonication, maximum lactase activity was achieved in 6 min in two *Lactococci* strains, 4 min in all strains of *L. delbruekii* subsp. *bulgaricus* and *S. salivarius* subsp. *thermophilus*, and in 12 min in *L. acidophilus* cultures. High sonication time for *L. acidophilus* culture as compared with other bacterial cultures may be an indication of a rigid cell wall of this organism. Once the maximum lactase activity was achieved, there was no decrease in the enzyme activity on further sonication. This was in contrast with the observations of Kilara and Shahani (1976), who reported a decrease in enzyme activity after 7 minutes of sonication of a yogurt culture. The decrease in enzyme activity in their study may have been due to an increase in temperature during sonication which would cause inactivation of the liberated enzyme, as observed in our preliminary experiments. Controlling the temperature is a crucial step during sonication of bacterial cultures.

The lactase activity of the four unspecified bacterial cultures before and after sonication is shown in Table 4-1 and that of two specific strains of *Lactobacilli* and *Lactococci* is given in Table 4-2. The unsonicated as well as sonicated cultures of *L. delbruekii* subsp. *bulgaricus* 11842 showed the highest lactase activity per gram of culture while *L. lactis* subsp. *cremoris* was the lowest. All the cultures contained approximately the same number of organisms per gram of culture. Lactase possessed by *L. lactis* subsp. *cremoris* is identified as ß-D-phosphogalactoside galactohydrolase (ß-P-gal) as opposed to *S. salivarius* subsp. *thermophilus* and *L. delbruekii* subsp. *bulgaricus* lactase which is known as ß-D-galactoside galactohydrolase (ß-gal) (Wong et al., 1987). *L. lactis* subsp. *cremoris* will hydrolyse lactose with ß-P-gal which will not split ONPG as a substrate. These organisms showed very little enzyme activity with ONPG substrate. The enzyme activity of the 2 specific strains of *Lactococci* measured with ONPG-6P substrate in the follow-up experiment was much higher. However, the activity was still less than that of the low activity strain of *L. delbruekii* subsp. *bulgaricus* 7994. Farrow (1980) and Wierzbicki and Kosikowski (1973) also observed negligible amount of lactase activity in *L. lactis* subsp. *cremoris* cultures. Upon sonication, there was about 5 - 8 times increase in the lactase activity of the three *L. delbrueckii* subsp. *bulgaricus* strains and of the unspecified strain of *L. acidophilus*, whereas *S. salivarius* subsp. *thermophilus* exhibited only about 1.5 times increase in the lactase activity. *L. lactis* subsp. *cremoris* strains 9596 and 14365 showed about 3- 5 times increases in the lactase activity after sonication.

4.3.2. Lactase in Broth Systems

To study the effectiveness of sonication on the release of free lactase, the cultures were grown in three different broths. The production of lactases by these organisms in the different broth systems varied, as shown in Table 4-3. *S. salivarius* subsp. *thermophilus* contained twice and 4 times more lactase activity than *L. delbruekii* subsp. *bulgaricus* in GAPT and LAPT broths, respectively. This was in contrast to the previous results from skim milk systems, where *L. delbruekii* subsp. *bulgaricus* showed higher lactase activity than *S. salivarius* subsp. *thermophilus*. The decrease in lactase activity of *L. delbruekii* subsp. *bulgaricus* and *L. acidophilus* grown in LAPT broth as compared to GAPT broth could have been due to inhibition of the enzyme activity by galactose (Kilara and Shahani, 1976). In all the organisms studied, a substantial part of the lactase was bound to the cell-debris. *L. acidophilus* did not possess much enzyme activity in cell-free medium.

4.3.3. Properties of Crude β-galactosidase

Data indicating the optimum pH and temperature conditions of the crude bacterial enzymes of *L. acidophilus*, *L. delbruekii* subsp. *bulgaricus*, and *S. salivarius* subsp. *thermophilus* are shown in Fig 4-3 and 4-4. In all three cases, the optimum pH was in the 6.0-7.0 range and the optimum temperature was 55°C. Crude lactases isolated from *L. delbruekii* subsp. *bulgaricus* 11842 exhibited similar pH and temperature optima (data not shown). The optimum temperature and pH conditions of *S. salivarius* subsp. *thermophilus* lactase were comparable to the previous reports (Somkuti and Steinberg, 1979; Ramana Rao and Dutta, 1981; Greenberg and Mahoney, 1982).

4.3.4. Effect of Acidic Conditions on Survival of the Microorganisms and their Lactases

The number of survivors of the four bacterial cultures during 2 h of incubation decreased at all pH conditions (Table 4-4). The viable counts decrease was substantial especially at pH 1.5 and 2.5. *L. acidophilus* showed the highest survival followed by *L. delbruekii* subsp. *bulgaricus* and *S. salivarius* subsp. *thermophilus* or *L. lactis* subsp. *cremoris*. Better survival of *L. acidophilus* as compared to *L. delbruekii* subsp. *bulgaricus* has been observed earlier by Conway et al. (1987). The higher survival rate of *L. acidophilus* and its 3 times higher sonication time may both indicate a more resistant cell wall material as compared to the other organisms tested. The viable populations decreased steadily at all 3 pH trials; after 2 h of incubation at pH 1.5, all sample populations decreased by about 6 log cycles. However, even at the pH 1.5 there were about 10^2-10^3 survivors after 1 h in all cultures. This was in agreement with the findings of Hood and Zottola (1988) for *L. acidophilus*.

The effect of incubation in acidic conditions on ß-gal activity of unsonicated *L. delbruekii* subsp. *bulgaricus* culture is shown in Fig 4-5. The enzyme activity decreased at all pH conditions studied. Incubation at pH 1.5 resulted in the most rapid loss of enzyme activity in 1 h; only small amount of activity remained after 3 h of incubation. Similar trends were observed for the enzyme activities of other cultures (data not shown).

Acidification of sonicated culture resulted in rapid and permanent loss of enzyme activity; only trace amounts of measurable activity remained after 1 h of incubation at all pH levels. When the pH of the sonicated samples after incubation was adjusted to 7.0, the enzyme activity was not restored, indicating permanent inactivation. This indicated that the microbial cell membrane, cell wall, or both may aid in protecting the ß-galactosidase from acid denaturation. The irreversible deactivation of the neutral lactases in acidic pH range is known to occur (Savaiano et al., 1984; Jackson and Jelen, 1989).

4.4. CONCLUSIONS

The optimum pH conditions of the crude lactases of unspecified strain of *L. delbruekii* subsp. *bulgaricus*, *L. delbruekii* subsp. *bulgaricus* 11842, *L. acidophilus*, and *S. salivarius* subsp. *thermophilus* were all in the neutral pH range, and the optimum temperature in all cases was about 55°C. The enzyme activity decreased with decreasing pH; at pH 3.5 or below the free enzymes were permanently inactivated while the enzyme activity of unsonicated cultures decreased less rapidly. Sonication time to release ß-gal was the highest for *L. acidophilus*; this organism also survived better than the others under acidic conditions, indicating the possibility of effective protection against adverse environment conditions such as found in the human gastrointestinal tract. *L. delbruekii* subsp. *bulgaricus* 11842 possessed considerably more ß-galactosidase activity than two specific strains of *Lactococci*, *L. acidophilus* or *S. salivarius* subsp. *thermophilus* especially in the skim milk system; the survival of the *L. delbruekii* subsp. *bulgaricus* in acidic conditions was also satisfactory. Although *S. salivarius* subsp. *thermophilus* contained the highest total lactase activity in broth systems, its activity in skim milk was much less pronounced. Cultures of *L. lactis* subsp. *cremoris* showed negligible amount of ß-gal activity with ONPG substrate, however, the activity of 2 specific strains of *L. lactis* subsp. *cremoris* was clearly demonstrated with ONPG-6P substrate.

4.5. TABLES AND FIGURES

Table 4-1. Lactase activity of four unspecified strains of bacterial cultures before and after sonication.

Organisms	Lactase activity			
	Unsonicated		Sonicated	
	$\bar{X}$	S.D.	$\bar{X}$	S.D.
	---(µmole o-nitrophenol/min.g culture)---			
L. delbruekii subsp. *bulgaricus*	0.38	0.02	1.63	0.13
S. salivarius subsp. *thermophilus*	0.21	0.05	0.35	0.02
L. acidophilus	0.14	0.02	0.85	0.04
L. lactis subsp. *cremoris*	0.07	0.01	0.09	0.02

Table 4-2. Lactase activity of two specific strains of *L. delbrueckii* subsp. *bulgaricus* and *Lactococcus lactis* subsp. *cremoris*, before and after sonication.

Organisms	Lactase activity			
	Unsonicated		Sonicated	
	$\bar{X}$	S.D.	$\bar{X}$	S.D.
	----(µmole ONP/min.g culture)----			
L. delbrueckii subsp. *bulgaricus* 11842	0.49	0.03	4.22	0.14
L. delbrueckii subsp. *bulgaricus* 7994	0.16	0.04	1.33	0.11
Lactococcus lactis subsp. *cremoris* 14365	0.10	0.01	0.31	0.02
Lactococcus lactis subsp. *cremoris* 9596	0.15	0.02	0.75	0.07

Table 4-3. Lactase activity of cultures grown in broth

Organisms and growth medium	Lactase activity		
	Bound	Free	Total
	---(µmole o-nitrophenol/min.g dry cell weight)---		
S. salivarius subsp. *thermophilus*			
MRS broth	262	108	370
GAPT broth	340	150	490
LAPT broth	365	159	524
L. delbruekii subsp. *bulgaricus*			
MRS broth	269	52	321
GAPT broth	225	23	248
LAPT broth	120	16	136
L. acidophilus			
MRS broth	166	11	177
GAPT broth	56	2	58
LAPT broth	21	1	22

Table 4-4. Survival of lactic acid bacteria under acidic condition

Organisms	pH	Time of incubation (h)		
		0	1	2
		------Log10 CFU/ml------		
L. delbruekii subsp. *bulgaricus*	3.5	8.32	6.14	5.86
	2.5	8.26	5.91	2.34
	1.5	8.02	2.53	1.46
L. acidophilus	3.5	8.96	6.59	4.52
	2.5	8.94	6.25	3.85
	1.5	8.11	3.08	1.70
S. salivarius subsp. *thermophilus*	3.5	8.82	4.10	2.56
	2.5	8.76	3.86	2.54
	1.5	7.88	2.57	0.60
L. lactis subsp. *cremoris*	3.5	8.45	6.17	4.48
	2.5	8.34	5.37	3.72
	1.5	8.02	2.30	1.57

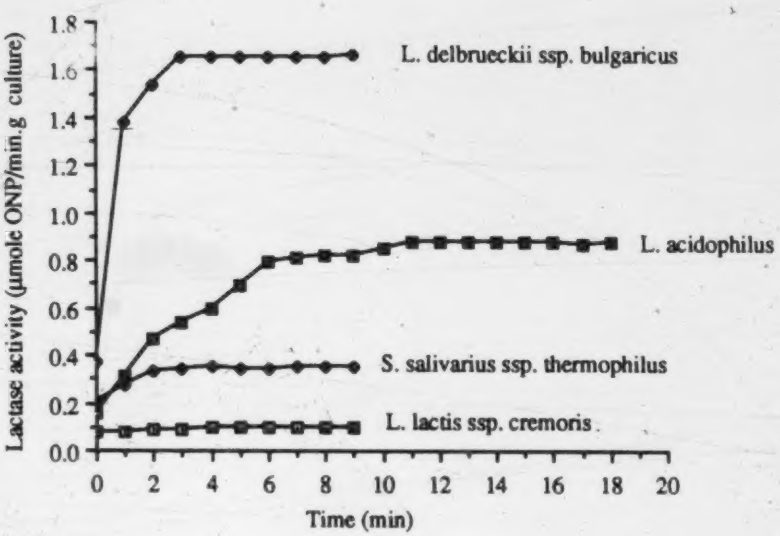

Fig 4-1. Effect of sonication on the release of cellular lactase enzyme activity in four bacterial cultures.

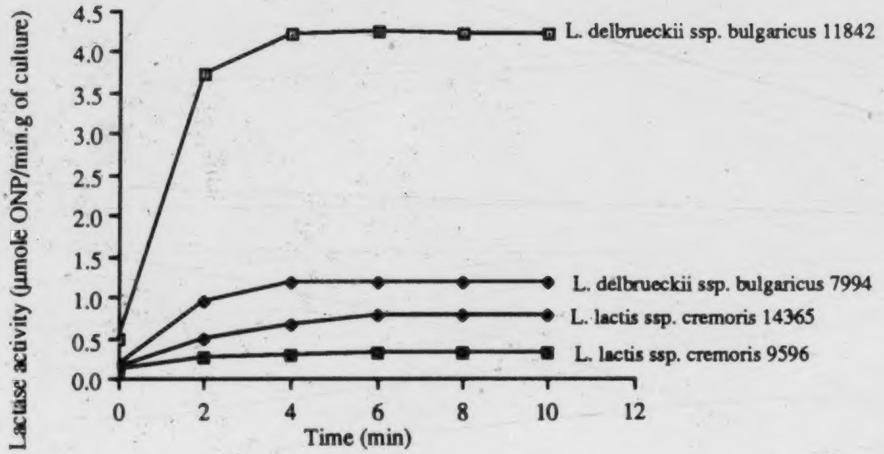

Fig 4-2. Effect of sonication on release of cellular lactase activity from four specific strains of *Lactobacilli* or *Lactococci*.

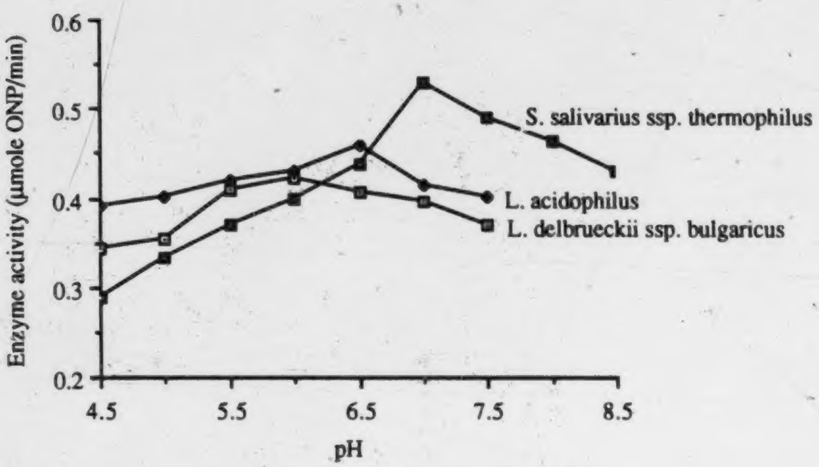

Fig 4-3. Optimum pH conditions of lactase enzymes isolated from unspecified strains of *L. delbruekii* subsp. *bulgaricus*, *L. acidophilus*, and *S. salivarius* subsp. *thermophilus*, measured at 37°C (crude enzyme preparations diluted 1500x for *L. delbruekii* subsp. *bulgaricus* and *S. salivarius* subsp. *thermophilus* and 250x for *L. acidophilus*).

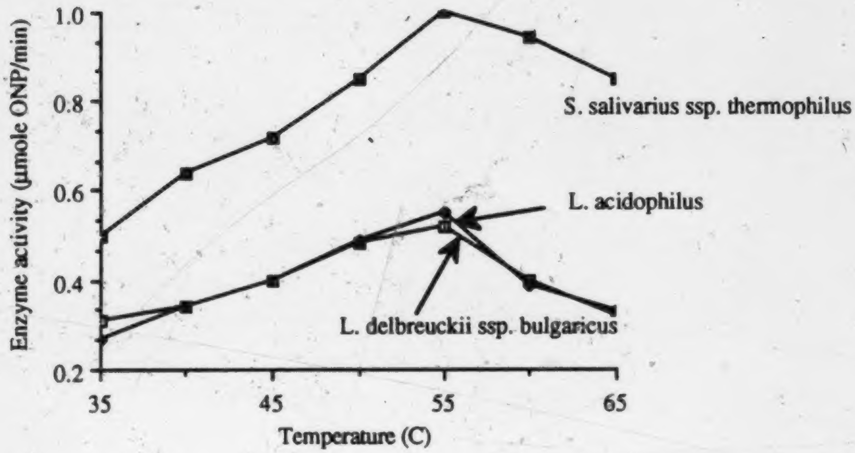

Fig 4-4. Optimum temperature of *L. delbruekii* subsp. *bulgaricus*, *L. acidophilus*, and *S. salivarius* subsp. *thermophilus* lactase enzymes (for pH optima and dilutions see Fig 4-3).

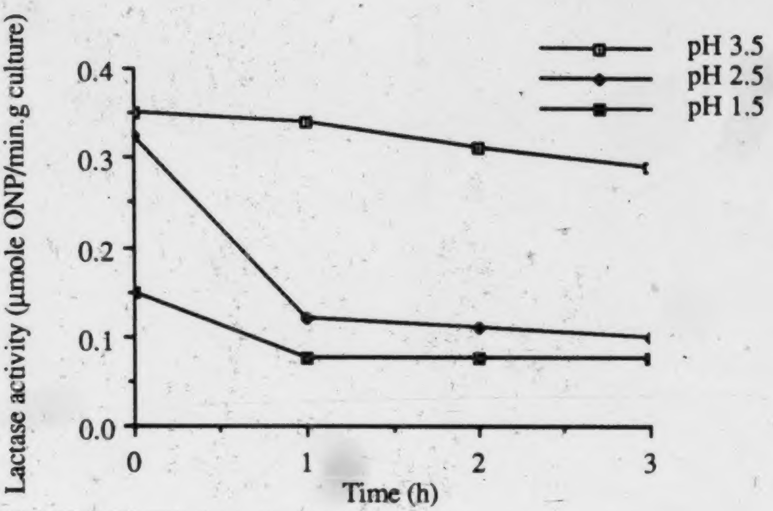

Fig 4-5. Effect of acidic conditions on the lactase activity of unsonicated *L. delbruekii* subsp. *bulgaricus* culture (unspecified strain).

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5. RENNET EFFECTS AND PARTITIONING OF BACTERIAL CULTURES DURING QUARG CHEESE MANUFACTURE[1]

5.1. INTRODUCTION

The traditional method of quarg cheese manufacture is based on coagulation of milk by bacterial cultures, such as *Lactococcus lactis* subsp. *cremoris* (formerly, *Streptococcus cremoris*) or *Pediococcus cerevisiae* (formerly, *Leuconostoc citrovorum*) (Kroger, 1980; Lang, 1980). A small amount of rennet is added following culture addition for better separation of protein coagulum from whey, for preventing excessive losses of casein fines, and for improved curd firmness (Lang, 1980; Jelen and Renz-Schauen, 1989).

The whey obtained from the traditional manufacturing process for quarg (also quark in German spelling) using pasteurized skim milk contains about 0.7% whey protein (Sheth et al., 1988). About 60% of the whey protein can be easily precipitated by high-heat treatment of the milk thus improving the quarg yield (Lang, 1980; Kroger, 1980; Mann, 1978; Hayes, 1987; Sheth et al., 1988). All new technologies in quarg processing are concerned with the recovery of whey proteins by high-heat treatment of either skim milk or whey (Kroger, 1980; Lang, 1980; Jelen and Renz-Schauen, 1989). However, heat treatment of milk is known to decrease the milk clotting effectiveness of the rennet addition in cheese manufacture (Jenness and Patton, 1959). Similarly, heating of the quarg milk may result in higher water retention in the quarg due to increased water-holding capacity of the denatured whey proteins (Jenness and Patton, 1959; Webb et al., 1980); this, in turn, may cause an increase of lactose content in quarg manufactured by this process to 4.0-4.5% as compared with 2-3% in traditionally made quarg (Jelen and Renz-Schauen, 1989). High-lactose quarg may be less suitable for lactose-intolerant consumers.

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The survival of *Lactobacillus acidophilus* and its ability to colonize the human gastrointestinal tract has been investigated by many researchers (e. g. Conway et al., 1987; Kim and Gilliland, 1983; Hood and Zottola, 1988). Yogurt culture organisms, such as *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus salivarius* subsp. *thermophilus* (formerly, *Streptococcus thermophilus*) contain more lactase activity than the *L. acidophilus* or the traditional quarg culture organisms such as *Lactococcus lactis* subsp. *lactis* (formerly, *Streptococcus lactis*), *Lactococcus lactis* subsp. *cremoris* (formerly, *Streptococcus cremoris*) and *Lactococcus lactis* subsp. *diacetylactis* (formerly, *Streptococcus diacetylactis*) (Shah and Jelen, 1990; Kilara and Shahani, 1976). The lactase activity present in yogurt may facilitate lactose digestion in the gastrointestinal tract and this may explain why yogurt is well tolerated by lactose-intolerant individuals (Kolars et al., 1984; Martini et al., 1987; Savaiano et al., 1984). Whether the residual lactose in the modern quarg could be tolerated as in yogurt has not been studied so far; this might depend on the content of the viable culture bacteria present.

Labneh (leben), a concentrated yogurt of around 24% total solids consumed in Middle Eastern countries, is manufactured by partial whey removal from yogurt (Tamime and Robinson, 1978; Robinson, 1987); in this regard, the product technology can be similar to that of quarg. The partitioning patterns for various microorganisms into the liquid and solid phase during the quarg curd separation are not known. The objectives of this study were: (i) to compare the use of yogurt, acidophilus, and buttermilk cultures in quarg production, (ii) to study the effectiveness of rennet addition in the conditions of highly heated milk, and (iii) to assess the partitioning of the culture organisms into the liquid and solid phases after quarg curd separation.

5.2. MATERIALS AND METHODS

5.2.1. Starter Culture

A buttermilk culture consisting of *L. lactis* subsp. *lactis*, *L. lactis* subsp. *cremoris*, and *Leuconostoc mesenteroides* subsp. *cremoris* (formerly, *Leuconostoc cremoris*) was obtained from a local dairy; freeze-dried *Lactobacillus acidophilus* culture was obtained from North Carolina State University, and pure cultures of *S. salivarius* subsp. *thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* were isolated from a commercial yogurt as previously described (Shah and Jelen, 1990). All the organisms were maintained in sterile 12% reconstituted non-fat dry milk. The buttermilk culture was grown at 22°C, *L. acidophilus* and *S. salivarius* subsp. *thermophilus* cultures at 37°C, and *L. delbrueckii* subsp. *bulgaricus* at 43°C, for 18 h. All the cultures were grown in stationary conditions and were transferred successively at least three times before use.

5.2.2. Processing

Quarg was produced using 500-mL batches of pasteurized homogenized 2% butterfat milk according to the general procedure (Fig 5-1) described earlier (Sohal et al., 1988). Two batches of milk were heat-treated at 85°C for 30 min and then cooled to 31°C, while two additional batches were tempered to 31°C without any heat treatment. All four batches of milk were inoculated with 2% buttermilk starter culture and incubated at 31°C. Microbial rennet from *Mucor miehei* (Miles Laboratories Inc., 32 Proudfit St., Madison, WI 53701) was diluted 20 times in distilled water and 2 mL of the solution added to one of the unheated and one of the heat- treated milk batches after 1 h of incubation, to obtain the rennet addition level of 0.2 mL/1000 mL milk. The incubation was carried out until pH 4.5 was reached, as determined by a pH meter Model 320 (Fisher Scientific Co.). Whey was separated by centrifuging the fermented milk at approximately 2000x g for 10 min in 500-mL cups using an International Centrifuge Model BE (International Equipment Co., 1284 Soldiers Field Road, Boston 35, MA) at room temperature (about 22°C).

The same procedure was followed for making quarg with the yogurt culture except for the incubation at 43°C, while two different heat treatments of the milk (85°C for 1 h, or 121°C for 15 min at 15 psi) and incubation at 37°C were used for quarg made with the acidophilus culture.

In a separate experiment, heat-treated milk (85°C for 30 min) was cooled to 43°C, inoculated with 1% cultures of *S. salivarius* subsp. *thermophilus* and *L. delbrueckii* subsp. *bulgaricus* and incubated at 43°C until the pH reached 4.5. Whey was separated by filtration using 3 layers of cheese cloth. Partitioning patterns of viable organisms were determined for all experimental products.

5.2.3. Enumeration of Microorganisms

The numbers of viable organisms in the fermented product prior to whey separation, in the separated whey, and in the quarg, were determined by plating serial 10-fold dilutions in 0.1 M phosphate buffer, pH 7.0. Aliquots of 1 mL were plated on MRS agar (Difco Laboratories, Detroit, Michigan) for enumeration of *S. salivarius* subsp. *thermophilus*, *L. delbrueckii* subsp. *bulgaricus*, and *L. acidophilus*, or on Difco-APT agar for enumerating the total buttermilk culture organisms. Duplicate plates were incubated anaerobically using BBL GasPak systems (BBL Microbiology Systems, Division of Becton, Dickinson, and Co., Cockeysville, MD 21030) at 31°C for 48 h for the buttermilk culture organisms, or at 37°C for 72 h for *S. salivarius* subsp. *thermophilus*, *L. delbrueckii* subsp. *bulgaricus*, and *L. acidophilus*.

5.2.4. Chemical and Sensory Analyses

Total solid contents of all quarg and whey samples were determined using an automated microwave oven balance (C E M Corp., P. O. Box 200, Matthews, NC 28106). The official Kjeldahl method (AOAC, 1980) was used to measure the nitrogen content (N) in all the samples; the total protein content was obtained as N x 6.38. Sensory evaluation of the coded quarg samples was performed in a laboratory setting within 1-3 days after manufacture by two trained judges, using an adapted cottage cheese score card as per the ADSA dairy judging methodology (Tobias, 1976). The purpose of the tasting trials was to note any obvious textural changes and off-flavors, especially bitterness.

Unless otherwise indicated, all the experiments and analyses were repeated twice. The results shown are averages of all available replicates.

5.3. RESULTS AND DISCUSSION

The yields of the various quarg and whey batches obtained from 500 mL milk are given in Table 5-1. As expected, quarg made from heat-treated milk showed an increase in the yield over that made from unheated milk, presumably due to the retention of the heat-denatured whey proteins and increased water-binding capacity of the ß-lactoglobulin-κ-casein complex (Webb et al., 1980; Jenness and Patton, 1959; Mann, 1984). Heat treatment of milk resulted in a decrease in the protein content of whey indicating denaturation and subsequent recovery of the whey protein with casein during the processing of quarg. When rennet was added to either heated or unheated milk, there was a substantial decrease in the uncorrected yield of quarg. Rennet increased the curd firmness through increase in total solids and more efficient removal of whey, especially in the quargs made from unheated milk. This appears to be an indication of the lower effectiveness of rennet in high-heat-treated milk. Impaired aggregation properties of the rennet-converted κ-casein micelles covered by denatured whey proteins are known to cause poor rennetability (Hooydonk et al., 1987).

Using the centrifugal separation method, the addition of rennet did not have a significant effect on the corrected yield (18% solids) as compared to the quarg made without rennet. Some casein fines were observed in the whey obtained from quarg produced without rennet treatment, but the nitrogen content of the whey (N x 6.38) was slightly lower as compared to wheys from the renneted milk, probably due to the proteolytic activity of the rennet. In general, as the uncorrected yield of quarg decreased the total solids and protein contents increased (Table 5-1) as a result of the firming effect of rennet. The addition of rennet to the heat-treated milks seemed to slightly increase the coagulation time for most cultures used.

The incubation times required to reach pH 4.5 varied from 3.8-4.6 h for yogurt, 5.8-6.3 h for buttermilk, and 17.4-22.2 h for acidophilus cultures (Table 5-2). Yogurt culture organisms are known to be fast acid producers compared to buttermilk cultures (Tamime and Robinson, 1988). It was not possible to make quarg from unheated milk using acidophilus culture because of sweet curdling of the milk before sufficient acidity developed. The *L. acidophilus* bacteria grew very slowly as reported previously

(Robinson, 1987) requiring more than 22 h for the pH to reach 4.5. For proper pH development, the milk had to be sterilized by autoclaving at 121°C for 15 min at 15 psi. This decreased the incubation time to 18 h from 22 h for milks heated at 85°C for 1 h. However, pronounced browning occured as a result of the autoclaving treatment, thus rendering the appearance of the final quarg unacceptable.

The partitioning of the culture microorganisms after the centrifugal whey separation is also shown in Table 5-2. In general, the quarg contained higher concentrations of microorganisms than the fermented milk, possibly as a result of the higher solids content. On the average, the fermented milk contained, 10^7-10^8 cfu/g, the quarg 10^8-10^9 cfu/g, and the whey 10^6-10^8 cfu/g. A similar partitioning pattern of the culture organisms was observed in the gravity filtration method (Table 5-3).

Since the whey contained a significant number of viable organisms, it could be suitable for making whey beverages for therapeutic purposes if secondary pasteurization would not be applied. It has been suggested that for Lactobacillus to have a therapeutic effect the minimum viable cell concentration should be 10^5/g (Robinson, 1987). All the experimental whey samples contained more than 10^5 viable organisms/g of whey.

The main sensory defects observed in the quarg products prepared with the three types of cultures are summarized in Table 5-4. Some of the quargs manufactured with all three cultures from milk using rennet were bitter, probably as a result of the proteolytic activity during processing or storage. The occurence of bitter samples appeared higher in the quargs produced by using yogurt culture organisms as compared to the other quargs, possibly due to the incubation at 43°C customary for yogurt fermentation, which is also the optimum temperature for rennet activity. Proteolytic activity of rennet and starter cultures resulting in bitterness have been reported by

Lawrence et al. (1972), Edwards and Kosikowski (1983), and many others. Bitterness development in traditional quarg can be minimized by using lower levels of rennet (Sohal et al., 1988).

In comparison to samples without rennet, the firmness of the renneted quargs was noticeably higher, especially when made from unheated milk. Some of the firmest products were too rubbery, probably due to the very high total solids content. All the quargs prepared from milks using acidophilus cultures were flat in taste. The flavor of the labneh-type quarg was reminiscent of yogurt with excessive acetaldehyde, contributing to the overall sharply acidic taste.

5.4. CONCLUSIONS

Quarg made from regular unheated milk using yogurt and buttermilk cultures had consistency and other textural qualities comparable to the commercial standard product as available on the market, while flavor differed with the bacterial cultures. Addition of rennet to unheated milk resulted in substantial increase of quarg firmness; this was less pronounced in highly heated milk, probably due to the inhibitory effect of high heating on rennet activity. All quarg products contained higher concentrations of the bacterial cultures used in their manufacture in comparison to the respective fermented milk or the separated whey.

5.5. TABLES AND FIGURES

Table 5-1. Yield and composition of quarg and whey prepared by using three different cultures

Type of culture	Treatment	Actual Yield (g)		Corrected Quarg yield (18% T.S. basis) (g)	Total solids		Protein	
					------%------			
		Quarg	Whey		Quarg	Whey	Quarg	Whey
Buttermilk	No heat, no rennet	145.8	321.7	148.4	18.3	6.6	8.2	.7
	No heat, rennet	107.0	373.7	152.0	25.6	6.8	11.7	.8
	Heat, no rennet	178.9	288.5	181.0	18.2	6.6	7.4	.4
	Heat, rennet	163.7	327.8	183.7	20.2	6.7	8.5	.6
Acidophilus	Heat[a], no rennet	147.8	339.8	202.1	24.6	6.6	9.4	.5
	Heat, rennet	139.7	355.8	203.8	26.3	6.9	11.2	.8
	Heat[b], no rennet	185.9	269.4	198.0	19.2	6.7	7.8	.5
	Heat, rennet	164.6	290.9	200.1	21.9	7.2	9.3	.8
Yogurt	No heat, no rennet	122.6	358.3	161.9	23.8	6.8	10.4	.6
	No heat, rennet	82.8	388.1	159.8	34.7	7.0	15.0	.8
	Heat, no rennet	172.7	269.0	195.3	20.4	6.6	8.2	.4
	Heat, rennet	139.0	344.5	190.2	24.6	6.6	10.8	.5

[a] Heat treatment at 85°C for 1 hr

[b] Heat treatment at 121°C for 15 min at 15 psi (autoclaved milk)

Table 5-2. Partitioning of microorganisms and incubation time to reduce pH of milk to 4.5 during manufacturing of quargs using three different types of culture

Type of culture	Treatment	Viable organisms			Incubation time (hr)
		Fermented milk	Quarg	Whey	
		--------- Log CFU/g ----------			
Buttermilk	No heat, no rennet	8.23	8.58	7.29	6.2
	No heat, rennet	7.62	8.69	7.06	5.8
	Heat, no rennet	8.14	8.57	7.04	5.9
	Heat, rennet	8.18	8.48	6.99	6.3
Acidophilus					
	Heat[a], no rennet	8.20	8.59	6.20	21.8
	Heat, rennet	8.14	8.65	5.92	22.2
	Heat[b], no rennet	8.22	8.59	6.09	17.4
	Heat, rennet	8.23	8.60	6.21	18.2
Yogurt	No heat, no rennet	8.13	8.63	7.77	4.2
	No heat, rennet	7.98	9.05	7.90	4.3
	Heat, no rennet	8.42	9.03	7.44	3.8
	Heat, rennet	7.90	9.03	7.44	4.6

[a]Heat treatment at 85°C for 1 h; [b]Autoclaved milk

Table 5-3. Total solids content and bacterial counts in fermented milk, quarg and separated whey obtained by gravity filtration

Treatment	Bacterial Count (Log CFU/g)	Total Solids (%)
Fermented milk	8.47	11.26
Quarg	8.99	17.40
Whey	6.48	5.99

Table 5-4. Summary of main sensory defects observed in quarg prepared with three different types of cultures

Type of Culture	No. of Samples	Sensory Attributes	Defect	Frequency	
				Without Rennet	With Rennet
Yogurt	8	Flavor	Bitter	1	7
		Texture	Pasty	2	-
			Firm/rubbery	-	2
Buttermilk	6	Flavor	Bitter	-	1
		Texture	Pasty	2	-
			Firm/rubbery	-	4
Acidophilus	4	Flavor	Bitter	-	2
		Texture	Flat	2	2
			Mealy/grainy	2	-

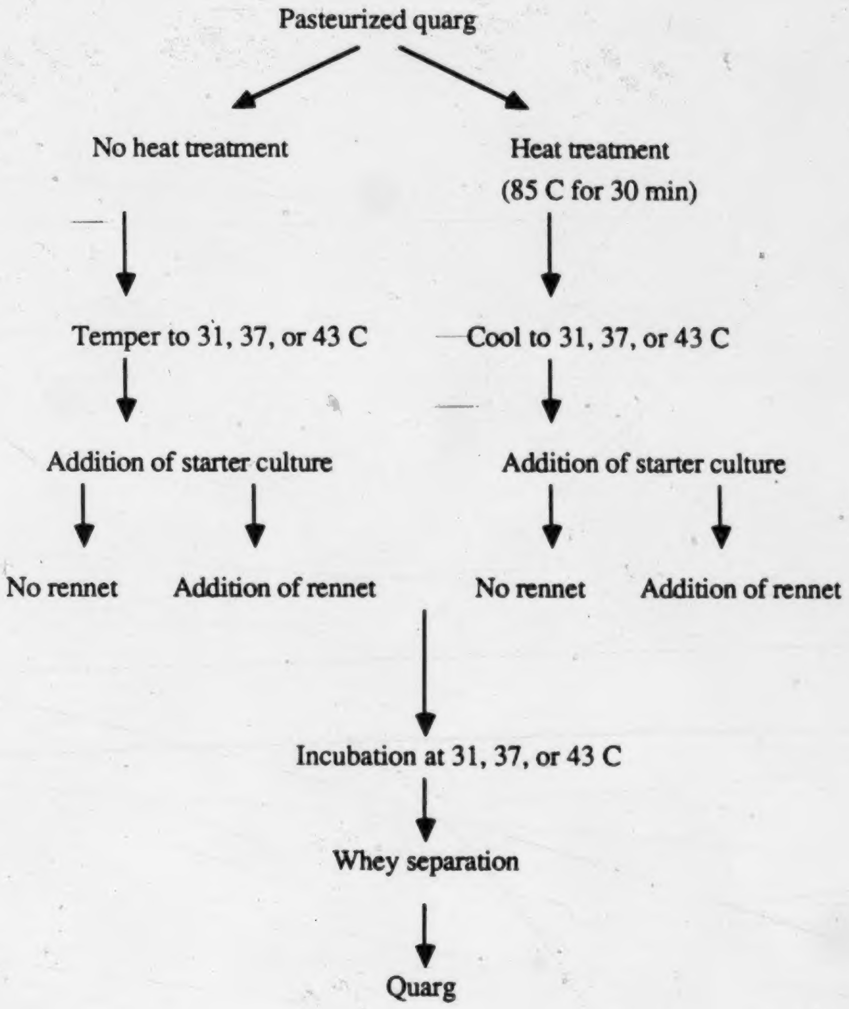
Pasteurized quarg
No heat treatment
Heat treatment
(85 C for 30 min)
Temper to 31, 37, or 43 C
Cool to 31, 37, or 43 C
Addition of starter culture
Addition of starter culture
No rennet
Addition of rennet
No rennet
Addition of rennet
Incubation at 31, 37, or 43 C
Whey separation
Quarg

Fig. 5-1. Flow diagram of experimental quarg production alternatives.

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6. LACTOSE ABSORPTION BY POST-WEANING RATS FROM YOGURT, QUARG, AND QUARG WHEY[1]

6.1. INTRODUCTION

Lactose, a disaccharide which occurs only in mammalian milk, is hydrolysed by a ß-galactosidase (lactase) enzyme localized on the brush border of epithelial cells of the small intestine (4, 13, 19). After hydrolysis, the resulting monosaccharides, glucose and galactose, are actively absorbed (3, 12).

Caucasians from northern European ethnic background and some African and Indian tribes maintain high intestinal lactase activity throughout life and can consume milk without any gastrointestinal discomfort (2, 19). In the majority of the world's population, the lactase activity falls to low levels in adulthood. This process is genetically programmed and is referred to as "primary lactase deficiency"(12, 30, 37). Lactase-deficient persons are unable to digest large amounts of lactose and may develop symptoms of lactose malabsorption such as diarrhea, bloating, and flatulence (13, 19, 34). These symptoms originate from fermentation of undigested lactose which enters the colon (19, 30).

Yogurt appears to be well tolerated by lactase deficient subjects (2, 7, 8, 17, 24, 29). Several studies (17, 20, 26, 29) indicated that the microbial ß-galactosidase of yogurt cultures could survive gastric passage and may facilitate the digestion of the residual lactose in the small intestine (22). Onwulata et al. (24) have shown that microbial endogenous lactase in yogurt is superior to a commercial lactase preparation in alleviating lactose intolerance symptoms. Ingestion of other fermented milk products such as acidophilus milk or cultured buttermilk is frequently associated with symptoms of lactose intolerance (17, 23, 27, 29), possibly because of the faster gastric passage of these liquid products and/or due to lower lactase activity of acidophilus (*Lactobacillus acidophilus*), buttermilk (e.g., *Lactococcus lactis* ssp. *cremoris*, *Lactococcus lactis* ssp. *lactis*) and other culture organisms as compared with yogurt culture organisms (*Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus salivarius* ssp. *thermophilus*) (16, 32).

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The ability of microorganisms to survive within the gastrointestinal tract and to hydrolyse lactose through action of their ß-galactosidase enzyme is still debated (28, 31). In a study by Schaafsma et al. (31) the significance of microbial ß-galactosidase activity from yogurt on lactose digestion in rats could not be confirmed. Gilliland (9) reported that the yogurt culture organisms do not survive or grow in the intestinal tract and therefore the organisms only serve as a source for the ß-galactosidase enzyme. On the contrary, Goodenough and Kleyn (7) have shown that a significant number of yogurt organisms can survive passage through the gastrointestinal tract. Acott and Labuza (1) and Shah and Jelen (32) have reported a significant number of yogurt culture organisms surviving conditions of a simulated gastric digestion.

Studies with lactase-deficient human subjects showed that pasteurized yogurt also can be well tolerated (29, 30) suggesting that there may be other factors involved besides microbial lactase activity as pasteurized yogurt should contain neither viable organisms nor lactase activity.

One such factor may be the composition of dairy foods (34). Foods containing higher total solids may result in a slower rate of gastric emptying leading to smaller amounts of lactose reaching the small intestine for more efficient hydrolysis and absorption (10, 25). In a study by Martini and Savaiano (21), lactose malabsorption symptoms were reduced with the ingestion of a lactose containing beverage along with a meal as compared with ingestion of the same beverage only, presumably due to delayed gastric emptying and/or the dilution effect of the meal in the gut.

Quarg, a fresh cheese of German origin produced with mesophilic lactococci starter organisms, is becoming popular among north American consumers. The lactose content of the modern quarg products may be higher than that of yogurt, however, the total solids are also substantially higher (14). Whether the residual lactose in quarg could be tolerated as in yogurt has not been studied so far.

The objectives of this study were: (1) to evaluate the lactose malabsorption effects of yogurt, quarg and liquid quarg cheese whey in post-weaning rats, and (2) to determine the survival levels of the microorganisms and/or their ß-galactosidase activity after passage through the upper gastrointestinal tract of the rat. Since rats become lactase deficient after the weaning period (31), they are suitable as an animal model for lactose malabsorption.

6.2. MATERIALS AND METHODS

6.2.1. Animals

Adult rats of the Sprague Dawley strain (Bio-Science Animal Services, University of Alberta, Edmonton, AB) were utilized in two separate experiments. The first experiment (replicated twice), was with ten female rats weighing 213 to 271 g, while ten male rats weighing 263 to 310 g were used in the second experiment. The rats were housed individually in cages and ad libitum water and rodent blox (Wayne Research Animal Diets, Brookfield, CT) were provided during a 48 h adjustment period. Light was controlled to provide 12 h of darkness and 12 h of light. Room temperature was maintained at about 24°C. Use of animals for this project was approved by Animal Policy and Welfare Committee, Faculty of Agriculture and Forestry, University of Alberta, Edmonton.

6.2.2. Lactose Malabsorption Test

After 48 h of adjustment, the rats were fasted for 12 h to eliminate the effect of the previous diet and about 0.7 mL blood sample from each rat was collected to determine the initial blood glucose level. The blood samples were collected in microtainer tubes (Becton Dickinson and Co., Rutherford, NJ) by cutting a small piece of the tip of the tail. The rats were first anaesthesized in a jar containing metophane (Pitman-Moore Ltd., Mississauga, Ont), an inhalation anaesthetic. The blood samples were transferred immediately to a refrigerator and serum glucose was determined within one hour of collection. The blood sample was centrifuged for 10 min using IEC Model HN-S ll centrifuge (International Centrifuge Co., 300 Second ave, Needham Heights, MA) at 6000 rpm. The clear serum was used for the glucose analysis.

After the initial blood glucose level was determined, the rats were provided with 25 mL of a 10% lactose solution. The feed bottles were removed after 2 h to determine the amount of lactose solution consumed and blood samples were taken for blood glucose determination to ascertain the level of the lactose intolerance. Lactose malabsorption symptoms such as diarrhea were also noted next day.

6.2.3. Experimental Foods and Feeding Methods

Five different dairy products evaluated in this experiment were: yogurt (Y), quarg prepared by using a yogurt culture (QY) and a buttermilk culture (QBM) and two types of wheys (WY and WBM) obtained from the quarg processing. Cultures (*Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus salivarius* subsp. *thermophilus*, for the QY and yogurt, or *Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *cremoris*, and *Leuconostoc mesenteroides* subsp. *cremoris*, for the QBM) were grown and quargs were processed as described previously (33). The yogurts were prepared according to the method of Kosikowski (18).

The numbers of viable organisms in the yogurt, quargs, and wheys were determined by appropriate plate count methods (33). The wheys contained lower number of viable organisms (10^6 cfu/mL) than the quargs (10^9 cfu/mL). To bring the number of organisms in the whey to the same level as the yogurt or the quarg, the organisms were grown separately; *Lactobacillus delbrueckii* subsp. *bulgaricus* in Lactobacilli MRS broth (Difco Laboratories, Detroit, MI) and all other organisms in Difco APT broth. The cells were harvested as previously described (32); the cell pellets were dissolved in sterile distilled water to a cell count of 10^{10} CFU/mL and added to the whey as required.

In the first experiment, after the lactose intolerance test the rats were fed each of the five dairy foods for one week in a sequence. The rats were given approximately 100 g of yogurt or quarg or 100 mL of whey prior to the lights being extinguished. No other feed supplement was used throughout the experimental period. Ad libitum water was provided in separate bottles in addition to the diets, except with the whey diet. The cups or bottles were removed the next day to determine the amount of food consumed in 24 h. Lactose malabsorption symptoms such as diarrhea were noted daily. After 7 days, blood samples from each rat were taken to determine blood glucose levels and the animals were weighed to determine weight change in each one week period. The animals were fasted for 12 h to eliminate the effects of the previous diet before starting them on another diet. The Y, QY, and QBM feeding periods were repeated using the same animals.

In the second separate experiment, five male rats were fed QY and another five were fed QBM following the determination of the base blood glucose level and the lactose intolerance test. Blood glucose levels of the rats were determined after one week feeding of the quargs. After 12 h fast, the rats which were on the QY diet were fed WY and those on the QBM diet received WBM for one week. The amount of food consumed was determined and the symptoms of diarrhea were noted daily. Blood glucose level was also determined after one week feeding of whey.

6.2.4. Viable Microorganisms and ß-galactosidase Activity in the Gastrointestinal Segments

On the final day of the first experiment, the rats were divided into 2 groups of five each and fasted for 12 h to eliminate the effects of the previous diet. One group of the rats was then provided with about 15 g of QY and another group with 15 g of QBM (except for one in each group which served as a fasted control) and allowed 30 min to consume the meal. Similarly, on the final day of the second experiment, the rats which were on WY diets were provided with about 15 g of QY and those which were on WBM diets were provided with about 15 g of QBM (except one in each group which served as a fasted control) after a 12 h fast. The animals were allowed 30 min to consume the meal as in the first experiment, and the cups were removed from all cages after the meal. In both experiments, one rat was sacrificed immediately after the completion of the meal and two more at 1 h intervals (i.e., 60 and 120 min after completion). The fifth rat was not used.

The rats were anaesthesized in a jar containing metophane and killed by decapitation. After surgical opening of the abdomens, the contents of stomach, duodenum, and jejunum were aspirated with a sterile 3 mL syringe after injecting 1 mL of sterile 0.1M phosphate buffer, pH 7.0 into tied-off segments and thorough mixing. Ten-fold dilutions of the aspirated contents were prepared in sterile 0.1M phosphate buffer and plated in duplicate on Lactobacilli MRS agar for yogurt culture organisms, or on Difco-APT agar for buttermilk culture organisms. The plates were incubated anaerobically using BBL anaerobic jars (BBL Microbiology Systems, Division of Becton, Dickinson and Co., Cockeysville, MD) at 37°C for 72 hours for yogurt culture organisms and at 31°C for 48 h for buttermilk culture organisms to determine the number of viable microorganisms in the gastrointestinal segments.

After aspiration of the samples for microbial assays, the tied segments of the gastrointestinal tracts (stomach, duodenum, jejunum) were removed from all sacrificed rats. They were kept in a frozen storage at -20°C for a few days and then assayed for lactase activity. The segments were cut apart with scissors and split longitudinally, and the entire contents were scraped into a tared aluminium dish and weighed again. The contents were then transferred to 15 mL polycarbonate centrifuge tubes with 3 mL distilled water. The extracts were mixed thoroughly with a sonicator (Artek Systems Corp., Farmindale, NY). One mL of the solution was used for an enzyme assay.

6.2.5. Lactase Assay

Ortho-nitrophenyl-ß-D-galactopyranoside (ONPG) or ortho-nitrophenyl-ß-D-galactopyranoside-6-phosphate (ONPG-6P) were used as substrates to measure enzyme activity from the gastrointestinal segments of QY and QBM fed rats respectively. The lactase activities of the gastrointestinal contents as well as of yogurt, quargs, or wheys were determined by incubating 1 mL of 10x diluted sample with 5 mL of ONPG or ONPG-6P at 37°C and measuring spectrophotometrically the amount of o-nitrophenol (ONP) released (32). One unit of lactase activity was defined as the amount of enzyme which liberated one µ-mole of ONP from ONPG or ONPG-6P per min per g of sample at 37°C.

6.2.6. Analytical Methods

Glucose contents in blood, quarg and whey samples were determined by the YSI sugar analyser Model 27 (Yellow Springs, OH). Lactose contents in all quarg, yogurt, and whey samples were determined by HPLC (5) using lactose as an external standard. Total solid contents in all yogurt, quarg and whey samples were determined with an automated microwave oven balance (CEM corp., P. O. Box-200, Matthews, NC). Student's t test was used to establish statistical significance of the differences in blood glucose from the base level after [illegible]g various dairy foods (36).

Unless otherwise indicated, all the experiments and analyses were repeated twice. The results shown are averages of all available replicates.

6.3. RESULTS AND DISCUSSION

6.3.1. Composition and Lactase Activity of the Experimental Diet

Table 6-1 summarizes the total solids, lactose contents, lactase activities and viable cell counts of the experimental dairy products. The total solids, lactose content and the viable cell counts of the quargs or the wheys were comparable; however, the type of culture organisms used for the two quargs and the resulting wheys were different. Thus, any differences in response upon feeding the two types of quarg or whey should be attributable to the type of culture organisms. Similarly, any differences caused by feeding yogurt, the quarg with yogurt culture (QY) or the resulting whey (WY) should be due to the differences in total solids contents of the products.

Y and QY contained higher enzyme activity as compared to QBM or the wheys. These results are in general agreement with earlier reports (16, 32), where no enzyme activity was observed in buttermilk culture organisms with ONPG as a substrate; however, in this study using ONPG-6P, cultured buttermilk showed some enzyme activity although much lower than the yogurt culture.

6.3.2. Lactose Malabsorption Symptoms

Absence of blood glucose level elevation and occurence of diarrhea were used as indicators of lactose malabsorption. Table 6-2 and Figs 6-1 and 6-2 show the effects of feeding different dairy foods on these lactose intolerance symptoms. Figs 6-1 and 6-2 show the blood glucose levels after 1 week feeding of the various dairy products in the two separate experiments. The increases in blood glucose level after feeding Y or quargs were significant (P< .01) as compared to the base blood glucose level. Blood glucose level was higher after feeding of quarg as compared to yogurt in both replicates of the first experiment. Similarly, blood glucose level was significantly higher after feeding quarg as compared to wheys. This could be the results of delayed gastric emptying of quarg as compared to the test lactose solutions or the whey, which would result in a slower delivery of lactose to the intestine for efficient hydrolysis by the lactase enzyme released from culture organisms and by the residual enzyme on the epithelial cells of the small intestine in the gut. As the glucose content in the quargs was determined to be about 25 mg/dL (data not shown), the rise in blood glucose level should have been due to the hydrolysis of lactose by the lactase enzyme into glucose and galactose in the gastrointestinal tract.

After feeding the whey, the blood glucose level did not increase and there was no statistically significant difference ($P>.05$) between the base level and that after feeding the whey. This is probably due to rapid gastric emptying of the whey. The consumption of total solids was much lower for the whey than for the quarg or the yogurt while the lactose amounts were comparable (Table 6-3) which may have contributed to the results observed.

Slightly soft stools were observed in a few rats during one week feeding of yogurt and QBM, but there were no symptoms of diarrhea with these products indicating good lactose tolerance. Feeding QY did not result in any incidence of soft stool, while after feeding the two types of whey, the rats displayed severe symptoms of diarrhea.

It is interesting to note that quarg prepared from buttermilk cultures (QBM) was tolerated as well as the yogurt or the quarg prepared from yogurt culture (QY). This is presumably due to the high total solids of quarg which should be handled by the stomach as a solid food (10, 25). In previous studies (29, 30) cultured buttermilk was not tolerated by lactose intolerant subjects. The total solids in quarg (QBM) are substantially higher than in buttermilk, hence, better tolerance of QBM as compared to cultured buttermilk could be due to its slower rate of gastric emptying, when the same bacterial cultures are used in the manufacturing process.

As the WY contained comparable number of viable organisms as Y and the ingestion of the WY whey resulted in the occurence of diarrhea in rats, it appears that the ingestion of viable yogurt organisms does not always result in efficient lactose digestion, and that the food consistency plays an important role. Fat containing liquid dairy product such as whole milk can be better tolerated than whey or skim milk as the fat delays gastric emptying (30). A liquid lactose solution, which is customarily used for lactose intolerance test, would pass through the gut much faster than a lactose-containing solid food as confirmed by our results with the two types of whey. Hence the practice of using liquid lactose solutions for a meaningful lactose intolerance test may be questionable.

Studies have shown that lactose intolerant individuals may still have some enzyme activity on the brush border of epithelial cells of the small intestine (13, 15). This residual lactase enzyme can presumably hydrolyse the lactose reaching the small intestine at a slower rate. If yogurt is being handled by the stomach as a solid food, this could explain why pasteurized yogurt (containing neither viable cells nor lactase activity) in the study of Savaiano et al. (29) was tolerated well by individuals diagnosed as being lactose intolerant by using a liquid lactose solution.

Lack of tolerance of both types of whey in the second experiment suggests that there was no carry over effect of the quargs. The rats, which were fed QBM or WBM diet, never received any yogurt culture which has been suggested as being able to survive gastric passage (7) and possibly even to colonize the gut (6, 11). Hence, the second experiment confirmed that the tolerance of QBM in the first replicated experiment was not due to any carry over of yogurt culture organisms from feeding yogurt or quarg (QY).

6.3.3. Viable Organisms in the Gastrointestinal Segments

Figs 6-3 and 6-4 show the average total viable cell counts of the gastrointestinal segments of QY and QBM fed rats at various intervals after the meal. The number of viable organisms from the fasted control rats was taken as representing the number of organisms before the feeding. After feeding the quarg, the viable cell count increased to reach the levels comparable to that of the quarg which contained 10^9 cfu/g. With the increased time after the feeding, the bacterial count decreased, possibly due to acid deactivation of bacterial cells in the stomach or due to the decrease in quarg contents as it passes into the gut. However, the cell counts 2 h after the meal were still about 10^4-10^5 cfu/g, suggesting that a significant number of microorganisms could survive the gastric digestion. These results appear to confirm earlier observations from simulated and in vivo gastric digestion experiments (1, 7, 32).

6.3.4. Lactase Activity

Table 6-4 shows the lactase activity measured in intestinal extracts after feeding QY and QBM. The lactase activity decrease after the meal may have been due to acid denaturation of the enzyme activity in the stomach and/or as a result of the gastric emptying process. However, the enzyme activity increased in the duodenum or jejunum 1 h after the meal, possibly as a result of the indigenous enzyme activity, increased bacterial concentrations present, or the action of bile acids on the culture organisms which may have altered the cell permeability and released the cellular enzyme. Gilliland and Kim (8) studied the effect of bile acids on culture organisms and observed a 2-3 fold increase in the lactase activity of the culture organisms in the presence of bile acids. The increase in enzyme activity was much more substantial with QY as compared to QBM, which may reflect the lactase activities of the two cultures (Table 6-1); in addition, any contribution of the indigenous lactase activity of the epithelial cells of the small intestine would have been missed in the test using ONPG-6P substrate.

Despite the lower enzyme activity in QBM as compared to QY, QBM was as well tolerated as QY, and the number of the microorganisms in the gastrointestinal tract after feeding QY or QBM was also similar (Figs 6-3 and 6-4). This seems to indicate that high levels of exogenous microbial enzyme activity (as in the case of yogurt culture organisms) are not necessary for efficient lactose digestion from a solid lactose-containing food.

6.4. CONCLUSIONS

Solid dairy foods containing lactose, such as Y, QY, and QBM used in this study may be well tolerated by lactose malabsorbers. Feeding of whey produced severe diarrhea in the experimental animals, and the blood glucose level dropped to the base blood glucose level after one week feeding of whey indicating poor lactose absorption. While previous reports claimed that cultured buttermilk is unsuitable for lactose intolerant subjects, quarg prepared from buttermilk culture organisms was tolerated by the rats as well as yogurt or quarg containing yogurt cultures. The number of viable organisms in the gastrointestinal tract up to 2 h after feeding QY or QBM was about 10^4-10^5 per mL. Assays of the gastrointestinal contents after quarg feeding showed sufficient enzyme activity after the gastric digestion. The presence of culture microorganisms, the exogenous lactase activity, and/or the slow gastric emptying of the yogurt and the quargs may all have facilitated the lactose digestion from the yogurt and the quarg. While this experiment was restricted to the rats, it seems reasonable that similar results can be expected from verification tests with lactase deficient humans presently underway.

6.5. ACKNOWLEDGEMENTS

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6.6. TABLES AND FIGURES

Table 6-1. Total solids, lactose content, lactase activity and bacterial count of the experimental dairy foods

Product[1]	Total solids (%)	Lactose (%)	Lactase activity (μMole ONP/min.g)	Plate Count
Y	11.45	3.72	.60	2.4×10^8
QY	22.19	3.75	.55	9.9×10^9
QBM	21.82	3.78	.11	5.8×10^9
WY	6.48	3.75	.07	8.0×10^{6b}
WBM	6.50	3.81	.04	6.9×10^{6b}

[1] Y=yogurt, Q=quarg, BM=buttermilk, W=whey.

[b] Final count adjusted to approximately 10^9 by addition of 1 mL of an appropriate cell suspension (containing 10^{10} cfu/mL) per 99 mL of whey.

Table 6-2. Lactose malabsorption symptoms after feeding different dairy foods[1]

Product [2]	Day 1	2	3	4	5	6	7
Y	-	-	+ (4 rats)	-	+ (2 rats)	-	-
QY	-	-	-	-	-	-	-
QBM	-	-	-	-	+ (5 rats)	+ (3 rats)	-
WY	+	+	++	+++	+++	+++	+++
WBM	+	+	++	+++	+++	+++	+++

-	No symptoms
+	Slightly soft stools
++	Very soft stools
+++	Watery stools, diarrhea

[1] Based on 20 observations per day for yogurt and 25 observations per day for other diets.

[2] Y=yogurt, Q=quarg, BM=buttermilk, W=whey.

Table 6-3. Average food intake by the rats in the course of the experimental trials

Experimental Food[1]	Food Intake[2] Average Daily Consumption (g)	SD	Range (g)	Average Amounts Consumed Daily[2] Total Solids (g)	Lactose (g)
Y	93.33	13.65	67.47-107.28	10.69	3.47
QY	74.89	5.63	69.81-82.16	16.62	2.80
QBM	72.94	3.65	67.61-75.88	15.91	2.76
WY	64.00	7.33	55.00-71.67	4.15	2.40
WBM	61.50	4.94	53.65-72.70	3.99	2.34

[1] Y=yogurt, Q=quarg, BM=buttermilk, W=whey.

[2] Based on 20 and 25 observations per day for 7 days for Y and other diets respectively.

Table 6-4. Lactase activity of intestinal extracts.

		Time after feeding the quarg (min)		
Type of quarg	Intestinal Contents	0	60	120
		------Lactase activity ------ (µmole ONP/min.g)		
Yogurt culture	Stomach	.54	.49	.42
	Duodenum	.36	.62	.85
	Jejunum	.29	1.36	1.49
Buttermilk culture	Stomach	.10	.09	.06
	Duodenum	.09	.11	.12
	Jejunum	.08	.13	.15

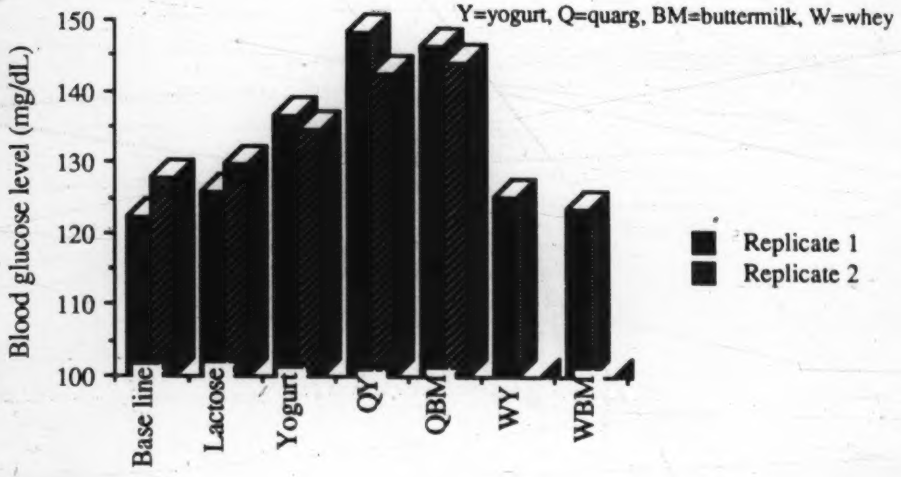

Fig 6-1. Blood glucose rise in rats after feeding experimental dairy foods in succession (experiment 1; results are averages of 10 female rats; range of S.D. from 9.57 to 19.16 with an average S.D. of 13.69).

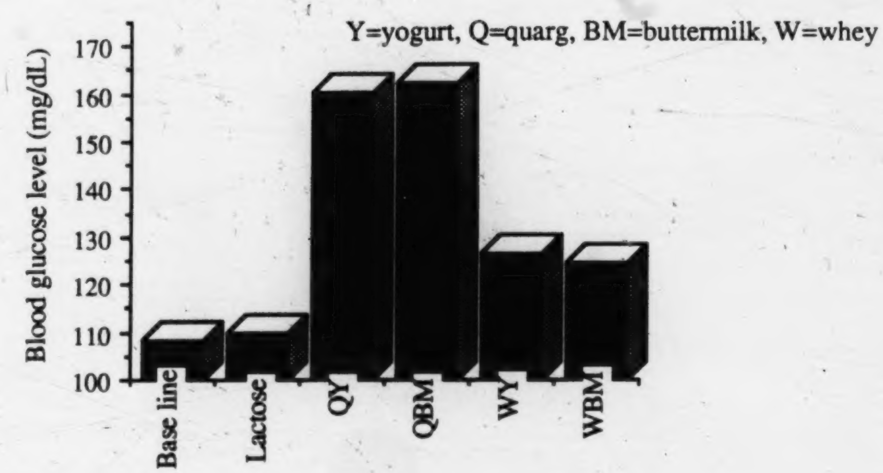

Fig 6-2. Blood glucose rise in rats after feeding experimental dairy foods in parallel (experiment 2; results for the dairy foods are averages of 5 male rats; range of S.D. from 7.72 to 13.89 with an average S.D. of 11.77).

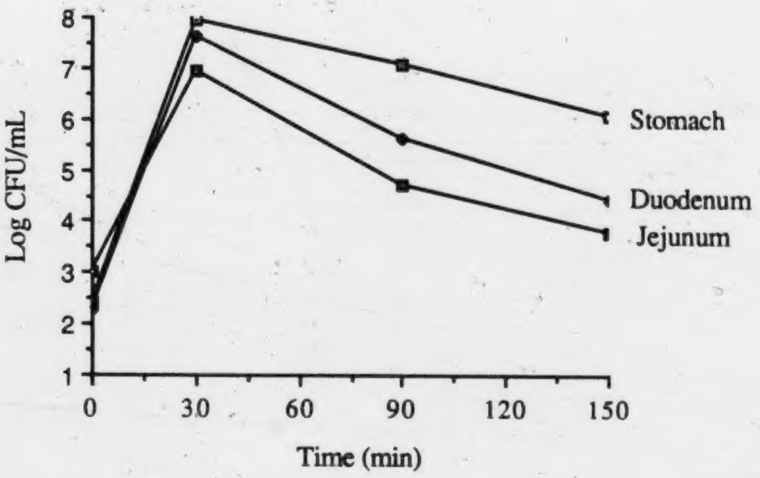

Fig 6-3. Bacterial count of gastrointestinal segments after feeding quarg produced with yogurt culture (averages of 2 different animals).

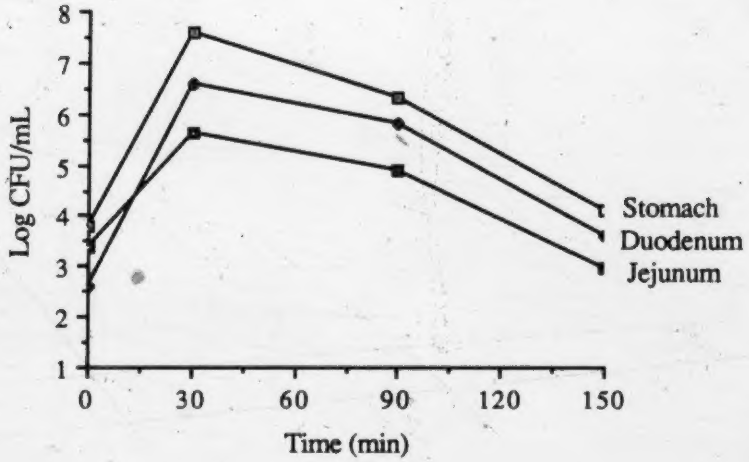

Fig 6-4. Bacterial count of gastrointestinal segments after feeding quarg produced with buttermilk culture (averages of 2 different animals).

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7. FOOD CONSISTENCY EFFECTS IN LACTOSE ABSORPTION BY LACTOSE INTOLERANT INDIVIDUALS FROM YOGURT, QUARG, PASTEURIZED QUARG, AND QUARG WHEY[1]

7.1. INTRODUCTION

Lactose, the main carbohydrate in milk, is composed of two monosaccharides, glucose and galactose. In infants, lactose is split by the ß-galactosidase enzyme located in the small intestine and the monosaccharides are directly absorbed in the blood (1 - 4). Lactose malabsorption due to intestinal lactase deficiency is prevalent in most adults who are not of northern European origin or where adults use little or no milk (5 - 8). About 70% of black Americans, and up to 95% of Asians are lactase deficient as compared to only 10-15% of US whites (6, 9, 10). When such lactase deficient persons ingest milk or lactose-containing dairy products, symptoms of lactose malabsorption such as flatulence, abdominal cramps, bloating or diarrhea may develop depending on the amount of lactose consumed. These symptoms result from fermentation of undigested lactose by microbial flora in the colon (2, 11, 12).

Cultured dairy products and lactose hydrolysed milk have been recommended for lactase deficient individuals (13 - 16). Among the cultured dairy products, yogurt seems to be particularly well tolerated by lactose malabsorbers (13, 17 - 19). Better tolerance of yogurt is attributed to higher ß-galactosidase activity in the organisms used for yogurt processing (normally, *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus salivarius* subsp. *thermophilus*) as compared to other lactic acid bacteria (12, 17, 20). However, some brands of yogurts are not effective in alleviating lactose malabsorption (21), and the significance of microbial lactase activity for lactose digestion is still debated (22). Other fermented milk products such as acidophilus milk or cultured buttermilk frequently cause malabsorption problems (18, 23), possibly because of the lower lactase activity of acidophilus (*Lactobacillus acidophilus*) or buttermilk culture organisms (*Lactococcus lactis* subsp. *cremoris* and *Lactococcus lactis* subsp. *lactis*) (24) or due to their fluid nature resulting in faster gastric passage (25).

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[1] A version of this chapter has been submitted for publication. N. Shah, P. Jelen, and R. N. Fedorak. 1990. Submitted to American Journal of Clinical Nutrition on Nov 8, 1990.

Food consistency appears to be an important factor responsible for efficient lactose digestion (20, 26, 27). In an animal model study (25), quarg, a soft cheese prepared from milk fermented by a buttermilk culture, was as well tolerated as yogurt or quarg with yogurt cultures by post-weaning rats, while fluid whey containing similar levels of yogurt culture organisms and lactose caused severe diarrhea. This would suggest that microbial lactase activity may not be the primary factor in alleviating lactose malabsorption.

The purpose of this study was to verify the results obtained from the animal model studies using lactase deficient human subjects. The main objectives were to (i) evaluate the lactose malabsorption effects of a solid dairy food represented by quarg, in comparison to semi-liquid and liquid foods, such as low total solids yogurt and quarg cheese whey, in lactose intolerant subjects, and (ii) to assess the relative importance of the type of culture, food consistency and residual (exogenous) enzyme activity in the dairy products in alleviating the lactose malabsorption symptoms.

7.2. MATERIALS AND METHODS

Pure cultures of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus salivarius* subsp. *thermophilus* were isolated and examined for purity as previously described (28). A buttermilk culture consisting of *Lactococcus lactis* subsp. *lactis* (formerly, *Streptococcus lactis*), *Lactococcus lactis* subsp. *cremoris* (formerly, *Streptococcus cremoris*), and *Leuconostoc mesenteroids* subsp. *cremoris* (formerly, *Leuconostoc cremoris*) was obtained from a local dairy. All the organisms were maintained in sterile 12% reconstituted non-fat dry milk. The buttermilk culture was grown at 26°C, *S. salivarius* subsp. *thermophilus* at 37°C, and *L. delbrueckii* subsp. *bulgaricus* at 42°C, for 18 h, respectively.

7.2.1. Processing of Experimental Foods

Five different dairy products were included in this study: quarg prepared by using a yogurt culture (QY) or a buttermilk culture (QBM), yogurt (Y), pasteurized quarg prepared with buttermilk culture (QP), and quarg cheese whey (WY) obtained from QY processing. The experimental foods were all prepared in the Food Science Department, University of Alberta.

Y, QY, QBM, and QP were all made from pasteurized 2% milk. The milk was heated to 85°C for 30 min. To produce the yogurt, the milk was inoculated with 1% each of *L. delbrueckii* subsp. *bulgaricus* and *S. salivarius* subsp. *thermophilus* cultures and incubated at 42°C until the pH reached 4.5 (29). No stabilizer or extra nonfat dry milk was added to increase the total solids or the viscosity of the product.

For manufacturing QY, the same cultures and incubation temperatures were used as in yogurt. Whey was separated using three layers of cheese cloth. QBM was prepared in the same way as QY except that a buttermilk culture (2%) and incubation temperature of 31°C were used. QP was prepared by heat treating (75°C for 30 min) milk fermented by a buttermilk culture to pH 4.5 followed by separation of the whey as in QY. The resulting quarg (QP) had higher total solids than QY or QBM. The separated whey was used to readjust the total solids of QP to the level of QBM or QY; the QP-whey mixture was homogenized with a polytron homogenizer (Brinkmann Instruments, Rexdale, Ontario).

Quarg cheese whey (WY) was the whey obtained from QY processing. Natural orange flavor (F & C, 1 Young Street, Suite 1801, Toronto, Ontario) was added at the rate of 0.05% for palatability.

7.2.2. Enumeration of Viable Microorganisms

The numbers of viable organisms in quargs, pasteurized quarg, yogurt, and whey were determined by standard enumeration techniques using 10-fold serial dilutions prepared in 0.1 M phosphate buffer, pH 7.0 (28). As shown previously (25), the whey contained lower number of viable organisms (10^7 cfu/mL) than the quargs (10^9 cfu/mL) or yogurt (10^8 cfu/mL) (Table 7-2). Final count in the whey was adjusted to the same level as yogurt or quarg as previously described (25).

7.2.3. Subjects and Experimental Design

The initial study group consisted of nine healthy subjects ranging from 32 to 39 years from Nepal, India, Malawi, and Canada. The characteristics of the subjects are given in Table 7-1. All subjects were informed about the purpose of the study and their written consents were obtained. The subjects were interviewed about their milk consuming habits and history of lactose intolerance. The project was approved by the Ethics Review Committee for Human Experimentation, Faculty of Medicine, University of Alberta, Edmonton. The study was conducted at Clinical Investigation Unit at the University of Alberta Hospitals.

A standard lactose tolerance test (LTT) was carried out first to identify those subjects that were lactose intolerant (lactose malabsorbers). Sufficient amount of saturated lactose solution to supply 50 g of lactose was given to each subject after an overnight fast (26). The lactose solution was ingested within 5 minutes. About 5 mL of blood sample was taken at the time of ingestion and then in 15 min intervals for 90 min for determination of glucose. The blood samples were centrifuged for 20 min at 8000 rpm using Beckman TJ-6 Model centrifuge attached to a Model TJ-R refrigeration unit (Beckman Instruments Inc., Palo Alto, CA 94304). The clear serum was used for the glucose analysis. Lactose malabsorption symptoms such as cramps, flatulence, bloating, and diarrhea were recorded by the subjects for 6 h following ingestion of the lactose drink. The persons with blood glucose elevation < 20 mg/dL and with symptoms of lactose malabsorption were classified as lactose malabsorbers (26). Only four lactose malabsorbers were selected for the main study (Table 7-1), in which each selected subject consumed each of the five products once a week. The subjects received one product at a given session. In this arrangements, the experiment lasted for 5 weeks. All the subjects received the foods in the same weekly order (QBM, QP, Y, QY, and WY). The subjects were asked to fast overnight and report the following morning to the Clinical Investigation Unit where they were hospitalized for about 2 hours. Each subject consumed 250 g of a given product. Before consumption of the food, a blood sample was taken (0 min); a ten min period was then allowed to consume the product, after which blood samples were taken at 15 min intervals for the next 90 min to determine the glucose response. The subjects recorded the symptoms of discomfort (if any) on a symptom record form for 6 h following ingestion. The rating was based on a scale of 0 to 5 where 0-no symptoms; 1-mild; 3-moderate; and 5-severe.

Analysis of variance and Duncan's New Multiple Range Test (30) were used to establish statistical significance of the differences in total blood glucose produced over a 90 min period following consumption of different dairy foods.

7.2.4. Analytical Methods

Lactose contents of the five products used were determined by Boehringer Mannheim Lactose/D-Glucose UV method (Boehringer Mannheim Gmbh Biochemica, 6800 Mannheim 31, W. Germany). The amount of lactose in 250 g of the experimental products was calculated and USP grade lactose powder was added as necessary to obtain equal lactose amount (15 g) in each product.

Total solids contents of the dairy products were determined by an automated microwave oven balance (CEM Corporation, P.O. Box-200, Matthews, NC) and their lactase activities were measured by incubating 1 mL of 10x diluted sample with 5 mL of o-nitrophenyl-ß-D-galactopyranoside-6 phosphate (for QBM or QP) or o-nitrophenyl-ß-D-galactopyranoside (for QY, Y and WY) at 37^{o}C and the amount of o-nitrophenol (ONP) released was measured spectrophotometrically (28). One unit of lactase activity was defined as the amount of enzyme which liberated one µ-mole of ONP per min per gram of sample at 37^{o}C. Glucose contents in blood samples were determined with YSI sugar analyser Model 27 (Yellow Springs Instruments, Yellow Springs, OH).

7.3. RESULTS AND DISCUSSION

7.3.1. Composition and Lactase Activity of Experimental Foods

The composition and viable cell counts of the different dairy foods used in this study are given in Table 7-2. Since the total solids and lactose contents in QY and QBM were kept similar, any lactose malabsorption effects observed after consumption should have been due to the differences in type of culture organisms used for the processing of the quargs. Pasteurization reduced the number of viable organisms from 1.75 x10^9 to 2.8 x10^2 cfu/mL in the pasteurized quarg (QP) and the measurable enzyme activity was completely eliminated. The tolerance of this product should be attributable to the food consistency and the residual lactase activity in the small intestine. And finally, the number of viable organisms and lactose contents were comparable in Y, QY and WY; however, the total solids in QY were much higher than either Y or WY. Thus, any differences in response after consumption of Y, QY and WY should be due to the differences in total solids contents of the products.

QY and Y contained higher enzyme activity than QBM, QP or the whey (WY). However, the exogenous lactase activity could be inactivated by the gastric low pH and thus be of little significance in alleviating lactose malabsorption. The irreversible inactivation of the neutral lactases in acidic pH condition is known to occur (18, 28, 31).

7.3.2. Lactose Malabsorption Symptoms

All the subjects who were classified as lactose malabsorbers by LTT (Table 7-1) declared that their regular consumption of milk or dairy products was very low because of discomfort. Studies (32, 33) have shown that individuals with reduced lactase activity could not consume one glass (250 mL) of milk without signs of intestinal distress.

Table 7-3 illustrates the lactose malabsorption symptoms as reported by the lactase deficient subjects after consuming each of the experimental dairy foods. Symptoms including abdominal cramps, bloating, and diarrhea were experienced by the subjects to a varying degree. There were no symptoms of lactose malabsorption reported with QY. Two of the four subjects reported symptoms of mild abdominal cramps 3 h after consumption of QBM and QP. It takes about 3 hours for complete digestion and absorption of a food (34) and it is possible that the symptoms of mild cramps after 3 hours may not have resulted from the experimental food since the subsequent food intake of the subjects was not controlled after the 90 min. Thus, it appears that QY, QBM, and QP were well tolerated by the lactase deficient individuals. This suggests that the microbial enzyme activities or the presence of viable cultures are not necessary for efficient lactose digestion.

Two of the four subjects reported symptoms of cramps also after consumption of yogurt. The total solids in yogurt was similar to milk or cultured buttermilk as no extra dry matter was added and the consistency was liquidy. Better tolerance of QY as compared to yogurt containing the same level of lactose, viable microorganisms, and lactase activity but higher total solids would indicate that the consistency of food might be more important than the exogenous lactase activity of the yogurt culture.

The number of viable organisms in WY and QY were similar; however, the ingestion of whey resulted in symptoms of lactose malabsorption in all the subjects while QY and Y were well tolerated. This could be the result of delayed gastric emptying of quarg (QY) as compared to the quarg whey (WY) which would result in a slow delivery of lactose to the intestine for efficient hydrolysis by the residual lactase enzyme located in the small intestine. This again suggests that the ingestion of viable yogurt organisms may not be necessary and that the food consistency together with the residual lactase activity in the small intestine may be more important for efficient lactose digestion.

The selected subjects apparently hydrolyzed some of the lactose which they consumed for LTT (Table 7-1) or in the experimental products (Table 7-4), probably by virtue of their own lactase enzyme contained in the epithelial cells of the small intestine (1, 35). This residual lactase activity may be the explanation for the tolerance of pasteurized yogurt (containing neither viable cells nor lactase activity but with 2% nonfat dry milk added to increase total solids) in the study of Savaiano et al. (18). This may also explain why, in our study, pasteurized quarg (containing no enzyme activity and a low number of viable microorganisms) was tolerated well by the lactose malabsorbers. In a study by Martini and Savaiano (27) three subjects of 12 showed symptoms of discomfort following consumption of skim milk with a meal as compared to 8 subjects of 12 with skim milk only. This could also be due to delayed gastric emptying and/or the lactose dilution effects of the meal in the gut.

Fig 7-1 shows the blood glucose elevation profiles for 90 min and Table 7-4 lists the maximum blood glucose increase as well as the total blood glucose accumulation over a 90 min period following the consumption of different dairy foods. All the experimental subjects showed a blood glucose increase < 20 mg/dL serum. Alm (13) also reported a small increase in blood glucose by lactase deficient subjects after consumption of low fat milk, yogurt or acidophilus milk. WY caused the highest rise in the blood glucose from the average base line for the 4 subjects, but the elevation in the blood glucose level was for a shorter duration, in accordance with the expected fast gastric passage for the whey. As a result, the total blood glucose accumulation in the 90 min period was much lower. The rise in blood glucose with QY, QBM, and QP were smaller as compared to WY, but the total blood glucose accumulation over the 90 min period for the more solid quarg products was significantly ($P<.01$) higher than for the more fluid Y or WY. Although the total blood glucose accumulation over the 90 min period with Y was slightly higher than that of WY, there was no statistically significant difference ($P>.05$) in the blood glucose accumulation between Y and WY. A food with high total solids such as the quargs should have taken much longer to pass from the stomach to the small intestine (26) resulting in a smaller rise in the blood glucose level but higher total glucose accumulation. Thus, the lack of a blood glucose rise from the base line value alone may not be a clear indication of the lactose intolerance status of an individual.

The subjects showed milder symptoms of lactose malabsorption with WY which contained 15 g of lactose as compared to that of 50 g lactose solution used for the LTT (Table 7-1). Fifty g of lactose is equivalent to about one litre of milk and is an unphysiological dose, while the lactose contents in all the experimental foods were only slightly higher than those of regular dairy products, including the quarg (36).

7.4. CONCLUSIONS

Lactose-containing dairy foods with higher total solids such as QY, QBM, or QP used in this study may be better tolerated by lactase deficient individuals than more fluid products with the comparable lactose content, including liquid yogurt or whey with yogurt cultures. Fluid yogurt produced the lowest blood glucose rise, low total blood accumulation, and symptoms of lactose malabsorption in two of the four lactose intolerant subjects. Better tolerance of pasteurized quarg (QP) as compared to yogurt or whey with yogurt cultures may be an indication that microbial enzyme activities or presence of viable culture may not be necessary for efficient lactose digestion from a high solids-containing food. Residual lactase activity in the small intestine might have been sufficient for the lactose digestion from QP. Consumption of QY did not produce any discomfort whereas whey (WY) with the same levels of lactose and yogurt bacteria resulted in symptoms of lactose intolerance in all the subjects, suggesting that food consistency seemed to play a major role in lactose digestion. The results suggest that persons who experience lactose intolerance symptoms due to lactase deficiency could consume solid dairy products containing moderate amounts of lactose without any major problems.

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7.6. TABLES AND FIGURES

Table 7-1. Characteristics of 9 subjects included in the preliminary screening for lactose malabsorption test.

Subject	Sex	Age	Weight (Kg)	Country of Origin	Blood glucose increase following 50 g lactose ingestion (mg/dL)	Symptoms following 50 g lactose ingestion
CM*	M	37	82	Malawi	12.5	Severe diarrhea
GS	M	36	79	India	62.0	None
HR	F	34	50	Canada	10.5	Severe diarrhea
KC	F	32	55	Nepal	7.3	Diarrhea
KD*	F	27	42	Nepal	8.0	Diarrhea
KH*	M	39	59	Nepal	12.5	Diarrhea
KU	M	72	37	Nepal	37.3	None
NS	M	35	71	Nepal	32.8	Slight gas
RC*	M	34	62	Nepal	8.0	Diarrhea

* selected for the main study

Table 7-2. Total solids, lactose content, lactase activity, and number of microorganisms in experimental dairy foods used for the main lactose malabsorption study

Product [a]	Total solids (%)	Lactose (%)	Lactase activity (U/g)[b]	Plate count	Amount fed	
					product [c] (g)	lactase (U/g)
QBM	22.56	3.68	0.09	1.75×10^9	255.80	22.5
QP	22.60	3.74	0.00	2.8×10^2	255.65	0.0
Y	11.50	3.46	0.56	3.6×10^8	256.35	140.0
QY	22.46	3.38	0.73	1.9×10^9	256.55	182.5
WY	6.18	3.38	0.05	4.4×10^7[d]	256.55	12.5

[a] Y=yogurt, Q=quarg, BM=buttermilk, P=pasteurized, W=whey

[b] One unit=µ mole o-nitrophenol released/min.g

[c] Lactose content in each product adjusted to 15 g

[d] Final count adjusted to approximately 10^9 by addition of 1 mL of an appropriate cell suspension containing (10^{10} cfu/mL) per 99 mL of whey

Table 7-3. Lactose malabsorption symptoms observed after consuming different dairy foods.

Products[a]	Symptoms		
	Bloat	Cramps	Diarrhea
QBM[b]	0	2	0
QP[b]	0	2	0
Y[b]	0	2	0
QY	0	0	0
WY[c]	2	1	1

[a] Y=yogurt, Q=quarg, BM=buttermilk, P=pasteurized, W=whey

[b] Two subjects reported mild cramps 3 h after consumption of the product

[c] All four subjects showed symptoms with WY

Table 7-4. Blood glucose rise and total blood glucose accumulation following consumption of various dairy foods (averages for 4 subjects; for baseline and peak values see Fig 7-1).

Product *	Maximum blood glucose rise (mg/dL)	S.D.	Total blood glucose accumulation ** (mg/dL x min)	S.D.
QBM	10.11	1.84	50.15[a]	6.35
QP	8.00	2.35	53.68[a]	14.48
Y	5.50	3.54	33.86[b]	9.09
QY	8.75	2.87	56.42[a]	12.67
WY	12.31	2.48	31.07[b]	2.42

* Y=yogurt, Q=quarg, BM=buttermilk, P=pasteurized, W=whey.

** Areas under the curves in Fig 7-1.

[a,b] Means with unlike superscripts differ ($P<0.01$)

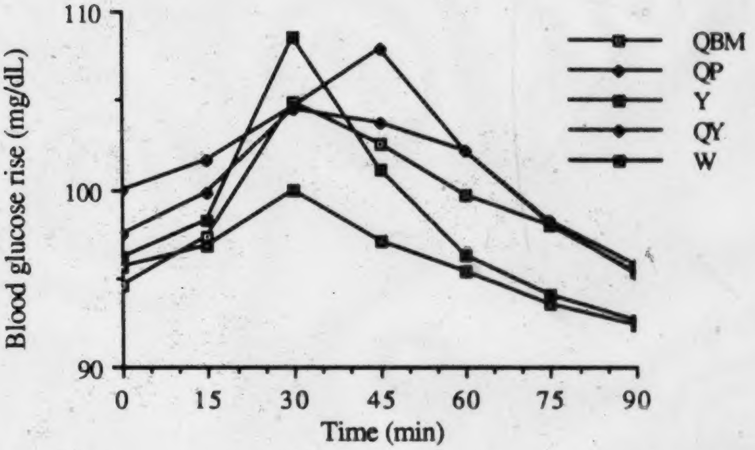

Fig 7-1. Effect of feeding different dairy foods on average blood glucose profile in lactose-intolerant subjects (n = 4).
(Y=yogurt, Q=quarg, BM=buttermilk, P=pasteurized, W=whey).

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8. SUMMARY AND CONCLUSIONS

The most important results obtained from these studies provide new information for further understanding of lactose absorption from dairy foods by lactose malabsorbers and offer a basis for design of whey containing foods suitable for lactose intolerant populations. The major findings summarized in this chapter appear to indicate that individuals who experience symptoms of lactose intolerance due to lactase deficiency could consume lactose-containing foods with high total solids without any symptoms of lactose malabsorption.

8.1. SUMMARY OF RESEARCH FINDINGS

The survey of several ethnic groups of Nepalese people indicated a high prevalence of lactose intolerance problem in Tharu ethnic group. In other ethnic groups, the incidence of lactose intolerance recorded in this study was low which may be due to low level of milk consumed by all ethnic groups. As milk production is scarce in Nepal, adult populations consume very little milk.

The properties and ß-galactosidase activities of several strains of lactic acid bacteria were studied (chapter 4). The optimum pH and temperature conditions of lactases isolated from *L. delbrueckii* subsp. *bulgaricus* 11842, unspecified strain of *L. delbrueckii* subsp. *bulgaricus*, *L. acidophilus*, and *S. salivarius* subsp. *thermophilus* were in the neutral pH range with optimum temperature of 55°C. The lactase enzyme activity of intact (unsonicated) cells decreased less rapidly at pH 3.5 as compared to other pH levels; however, there was rapid and permanent inactivation of the enzyme activity of the sonicated cells at this pH. Irreversible loss of enzyme activity of neutral lactases in acidic conditions has also been reported by Martini et al. (1987) and Jackson and Jelen (1989). This indicates that the bacterial cell wall protects the enzyme activity from acid denaturation. Yogurt is suggested to play a role in buffering the pH of stomach (Martini et al., 1987) and thus survival of yogurt culture organisms and their enzyme activities in the acid environment of the stomach is likely to be possible, while use of free lactase enzyme preparations or sonicated cultures in foods for lactose intolerant consumers would appear inefficient.

Among the lactic acid bacteria studied (chapter 4), *L. delbrueckii* subsp. *bulgaricus* 11842 possessed the highest lactase activity and unspecified strain of *L. lactis* subsp. *cremoris* the lowest. Cultures of *L. lactis* subsp. *cremoris* exhibited negligible ß-galactosidase activity with ONPG substrate; however, the enzyme activity of this organism with ONPG-6P was noticeable. The claim that *L. lactis* subsp. *cremoris* culture does not contain any lactase activity (Kilara and Shahani, 1976) and thus is unsuitable for lactose intolerant individuals is contradicted by this study.

Survival of lactic acid bacteria under acidic conditions was also studied (chapter 4). *L. acidophilus* survived better than *L. delbrueckii* subsp. *bulgaricus*, *S. salivarius* subsp. *thermophilus* or *L. lactis subsp. cremoris*. Sonication time to release ß-galactosidase activity was high for *L. acidophilus* indicating a rigid cell wall of this organism. *L. acidophilus* has been found to survive and colonize the gastrointestinal tract (Conway et al., 1987; Hood and Zotolla, 1988) probably by virtue of its resistant cell wall structure. Although *L. acidophilus* has been found to survive the gastric passage conditions, acidophilus milk, which is prepared using *L. acidophilus* cells, has been reported to be unsuitable for lactose intolerant individuals (Newcomer et al., 1983; McDonough et al., 1987; Onwulata et al., 1989). Use of *L. acidophilus* is advocated for therapeutic purposes (Robinson, 1987; Hitchins and McDonough, 1989).

Other lactic acid bacteria such as *L. delbrueckii* subsp. *bulgaricus*, *S. salivarius* subsp. *thermophilus*, and *Lactococcus lactis* subsp. *cremoris*, were also found to survive at lower pH levels; about 10^2-10^3 organisms out of 10^8 survived at pH 1.5 for 1 hr. The resident time for food in the stomach varies from 30 min to 1 hr and the pH of stomach, as reported by Martini et al. (1987), remains > 2.5 after consumption of yogurt; it is likely that a significant number of microorganisms and their ß-galactosidase activity could survive passage through the gastrointestinal tract.

Use of yogurt, buttermilk, and acidophilus cultures in quarg processing was compared and partitioning patterns of these culture organisms into quarg and resulting whey were studied (chapter 5). Quarg prepared from regular unheated milk using yogurt and buttermilk cultures had consistency and other textural qualities similar to the commercial product available on the market. Acidophilus culture was unsuitable for quarg processing as the milk had to be autoclaved for proper acid development and the incubation time was long (about 18 hr). Autoclaving milk imparted pronounced browning rendering the quarg unacceptable. All quarg cheeses contained higher number of bacterial cultures used in their manufacture as compared to the respective fermented milks or the separated whey.

The quargs and whey were used to assess the lactose malabsorption effects of solid and liquid foods using post-weaning rats (chapter 6). Feeding of yogurt (Y), quarg prepared from yogurt (QY) or buttermilk (QBM) culture for one week did not produce symptoms of diarrhea in rats indicating good lactose tolerance. Better tolerance of QY or QBM was possibly due to their high solid contents which would result in slower delivery of lactose to the intestine for efficient hydrolysis by the lactase enzyme released from cultures or the residual ß-galactosidase in the small intestine. Plating and assays of gastrointestinal contents after quarg feeding showed significant number of microorganisms and lactase enzyme activity surviving gastric digestion. This confirmed earlier observation from in vitro study that lactic acid bacteria and their lactases can survive gastric passage conditions (chapter 4). Although this information was based on rats, it appears reasonable that both the microorganisms and their lactases could survive the gastric digestion in humans as well. QBM contained lower exogenous ß-galactosidase enzyme activity than QY, however, both types of quargs were tolerated well by the rats. This indicates that high levels of exogenous enzyme activity such as found in yogurt organisms do not appear to be necessary for efficient lactose digestion from a solid-containing food. Feeding of whey with similar number of viable organisms and lactose content as Y or QY resulted in diarrhea suggesting that the presence of viable organisms and their lactases is not the primary causative agent for efficient digestion and absorption of lactose from solid foods.

Blood glucose level of the rats was significantly higher after one week feeding of quargs as compared to wheys. After feeding whey, the blood glucose level dropped to the base blood glucose level indicating poor lactose absorption. The results (chapter 6) indicated that the microorganisms and ß-galactosidase enzyme survived gastric digestion in the rat, however, they played a minor role in efficient digestion and absorption of lactose from foods with high solids. Food consistency appeared to be an important factor.

The results obtained from the rat studies were verified using 4 lactase deficient human subjects (chapter 7). QY was found to be well tolerated by lactose malabsorbers. Two of the four subjects showed symptoms of lactose intolerance with QBM, QP or yogurt. The number of viable organisms, and lactose contents were similar in quarg whey (WY), Y, and QY; however, WY produced symptoms of lactose malabsorption in all the subjects. Comparable tolerance of pasteurized quarg and yogurt suggests that viable culture organisms and their lactase activity are not necessary for efficient lactose digestion and that consistency of food plays a major role in satisfactory digestion and absorption of lactose to avoid problems. Lactase deficient individuals should have some residual enzyme activity in the intestine which may be responsible for hydrolysis of lactose from pasteurized quarg.

All the lactose deficient subjects showed a blood glucose increase < 20 mg/dL serum. Alm (1982) also observed a small rise in blood glucose level in lactose malabsorbers. The results suggest that the lack of a blood glucose rise < 20 mg/dL from the base line values alone can not be used as a basis for assuming lactose intolerance problems in lactose malabsorbers using a solid food. Although the rise in blood glucose with QY was < 20 mg/dL serum, this product did not produce any symptoms of lactose intolerance.

Ingestion of WY resulted in higher rise in blood glucose (although the rise in blood glucose level was < 20 mg/dL) than other experimental foods, however, the elevation was for a shorter duration; as a result, the total blood glucose accumulation in a 90 min period was lower. Foods containing high total solids such as quarg (QP, QY), resulted in slow gastric emptying causing smaller rise in the blood glucose level, however, the total accumulation over the 90 min period was significantly ($P<.01$) higher than that of WY.

WY containing 15 g lactose showed milder symptoms of discomfort as compared to 50 g lactose solution used for LTT (Table 7-1, chapter 7). The severity of symptoms of lactose malabsorption depends on the amount of unabsorbed lactose reaching the colon. The results indicated that lactase deficient persons could consume lactose containing dairy foods with high solids, such as quarg, without any symptoms. Quarg prepared with mesophilic cultures can be similarly tolerated as quarg made from yogurt culture. Even quarg pasteurized for longer shelf life and containing no viable bacteria or lactase activity probably could still be tolerated by lactose intolerant individuals.

8.2. APPLICATION OF RESEARCH FINDINGS

8.2.1. A Concept of a Nepali Food Containing Whey Suitable for Lactose Malabsorbers

Rice and corn meal, the main staple foods in Nepal, are usually cooked in water. Whey can be used for cooking rice or corn meal instead of water thus utilizing the nutrients from the whey. A solid food prepared from cornmeal commonly known as "dhinro" in Nepali, is a popular food in the hilly regions of Nepal. To process this food, cornmeal is added to boiling water and cooked by stirring until solidified. In an informal trial carried out at the conclusion of this project, cornmeal (1 part) was cooked in cottage cheese whey (6 parts). First of all, whey was brought to boil in a pan and cornmeal was added followed by mixing with a stirrer until a desired consistency was achieved. The total solids were 24.5%.

The whey - cornmeal based pudding was taste tested by an informal panel of 3 lactose malabsorber Nepali subjects who also participated in the previous study (chapter 7). Food consistency, taste, flavor, and overall liking were scored on a 9 point hedonic scale; 9 being the best and 1 being the worst. The average score was: consistency, 7.78; taste 3.31; flavor, 6.64; and overall liking, 4.3. General comments were that the product will taste better with some vegetables as is traditionally eaten in Nepal. The three Nepali subjects informally consumed 200 g of the product for the breakfast after an overnight fast and they did not report any symptoms of lactose malabsorption. This indicates a promising avenue for further developments, however, much additional work is required to test this product using a large number of lactose intolerant people.

8.2.2. A Conceptual Proposal for Industrial Process

The question of the way the whey produced in the hilly regions of Nepal could be utilized will be of great challenge. Sixteen out of 17 cheese processing units are located in the hills where the means of transportation is limited. Only one cheese factory is in the Terai zone and is assessible by roads. Besides these, there are some private dairies which process cheese. Most of the cheese plants are small and the amount of milk processed per day would range from 500 to 1000 litres, leaving behind approximately 450 to 900 litres of whey in each plant each day.

To overcome the accessibility problem in the hills, it is recommended that each plant utilize its own whey in processing corn meal-whey based pudding. No extra equipment will be required for processing this product as most of the dairy plants have ghee boilers which can be used for pudding processing as well.

Most of the dairies process dahi (a fermented milk product similar to yogurt) on a large scale. Dahi is usually packaged in earthenware pots. A similar packaging material can be used for the proposed product.

First of all, the product needs to be tested on a small scale and then a feasibility study of large scale production have to be carried out. As this product can be manufactured from inexpensive ingredients, the price per unit of the product will be affordable by the people. Corn meal is plentiful, inexpensive, and used as a staple food in the hilly regions where most of the cheese plants are located. Thus it seems feasible to manufacture the pudding type product for lactose intolerant people utilizing nutrients from the whey.

8.3. RECOMMENDATIONS FOR FUTURE STUDIES

Further studies towards better understanding of digestion and absorption of lactose by lactose intolerant individuals are still necessary. Before any whey-based food products are introduced in Nepal, further work is needed to ascertain the exact level of prevalence of lactose intolerance problems in the various Nepali ethnic groups by administering the standard lactose tolerance test.

Plate counts and enzyme assays of gastrointestinal contents of rats confirmed survival of lactic acid bacteria and ß-galactosidases after feeding two types of quarg (chapter 6). However, survival of these bacteria and their lactases remain to be verified in human subjects.

L. delbrueckii subsp. *bulgaricus* strain 11842 was found to possess the highest lactase activity among the lactic acid bacteria and the *L. delbrueckii* subsp. *bulgaricus* strains studied. Further studies are recommended to survey several other strains of *L. delbrueckii* subsp. *bulgaricus* for the presence of their ß-galactosidase enzyme levels to select the strains that are most beneficial for processing dairy products suitable for lactose intolerant consumers. Lactase enzyme released by sonication from the strains possessing high ß-galactosidase activity could be used to hydrolyse a portion of lactose in milk thus reducing the lactose content of the product (Shah and Jelen, 1990). A recent study from Japan (Toba et al., 1990) used sonication technique to produce lactose-hydrolysed fermented milk.

Individuals have varying levels of residual lactase enzyme in the small intestine by virtue of which each individual can hydrolyse different amount of lactose. As a result, subjects show varying degrees of tolerance for lactose. Additional studies are required to develop a reliable method to determine an individual's threshold for lactose for the occurence of symptoms both from solid and liquid type of foods as the thresholds for lactose tolerance may vary depending on the type and viscosity of food. This would help lactose malabsorbers know their threshold of lactose so that they could consume lactose containing dairy foods with less lactose than their limits.

Lactose tolerance test (LTT) and breath hydrogen test (BHT) are based on liquid lactose solution. A rise in blood glucose during LTT reflects the amount of residual lactase activity in the small intestine. Additional work is needed to establish a relationship between a rise in blood glucose level from foods containing high solids as a test to identify lactose malabsorbers. The results from the study on human subjects (chapter 7) suggested that blood glucose level does not rise >20 mg/dL in lactase deficient individuals even with the foods which do not produce symptoms of lactose malabsorption.

Breath hydrogen test measures the amount of unabsorbed lactose reaching the colon. There is a correlation between the amount of lactose fermented and hydrogen gas formed by colonic flora. In this study (chapter 7) breath hydrogen was not measured to monitor lactose malabsorption. Further studies may be useful to observe the amount of breath hydrogen produced, if any, from unabsorbed lactose from the yogurt, pasteurized quarg, or quarg produced from buttermilk culture.

The proposed hypothesis of this study was that solid foods result in delayed gastric emptying leading to slower delivery of lactose to the intestine for efficient hydrolysis. The actual gastric emptying times of solid food in comparison to liquid food as well as the amounts of unhydrolysed lactose, if any, remain to be measured.

The results on human subjects (chapter 7) are based on small number of observations. Additional studies are recommended using large number of subjects. Also, the study was based on 15 g of lactose (equivalent to approximately 325 mL of milk) which is a physiological dose. The standard lactose tolerance test is based on a lactose load of 50 g which is equivalent to about one litre of milk. Further work is required to evaluate whether quargs or other foods containing 25 g or 50 g lactose would be similarly tolerated.

Lactose intolerance appears to be a major problem in Nepal (Shah, 1990, unpublished data). It seems feasible to incorporate whey to foods for consumption by lactose intolerant people. Hopefully, the results from this study and the conceptual proposal for industrial process of a whey based pudding type food will contribute to solving malnutrition problem by utilizing valuable nutrients from the whey which is otherwise wasted in Nepal.

8.4. REFERENCES

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